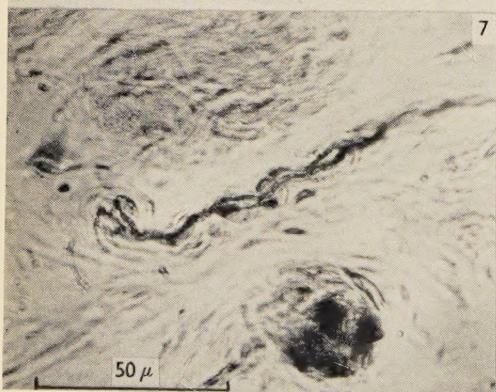
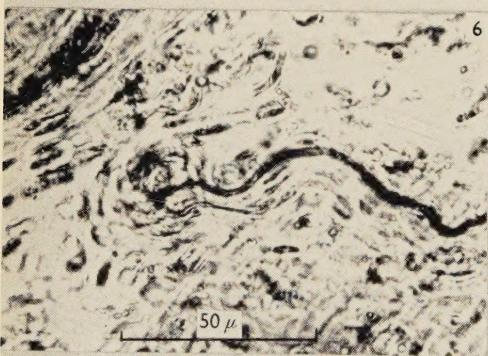
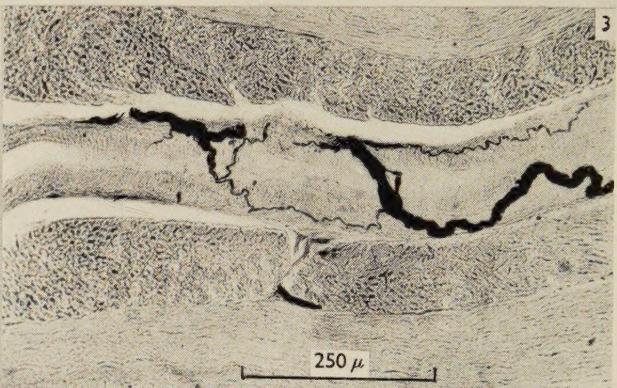
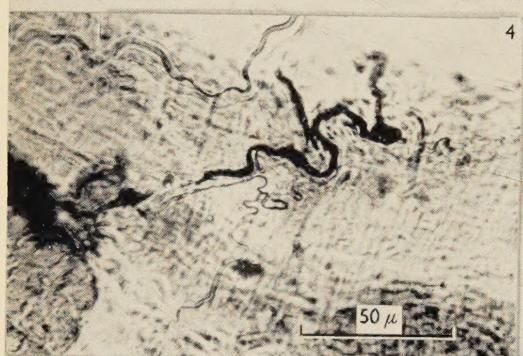
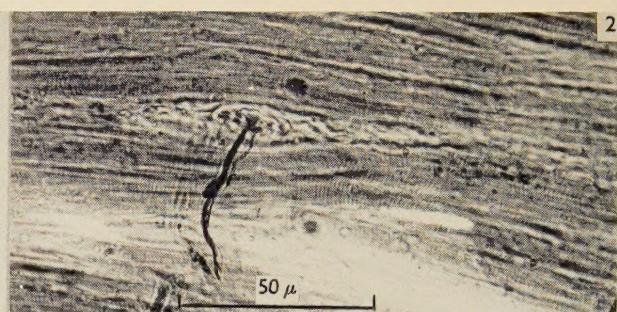
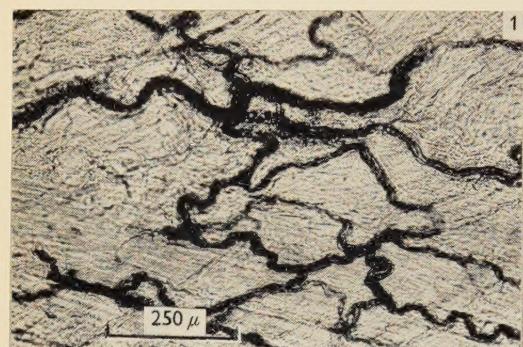
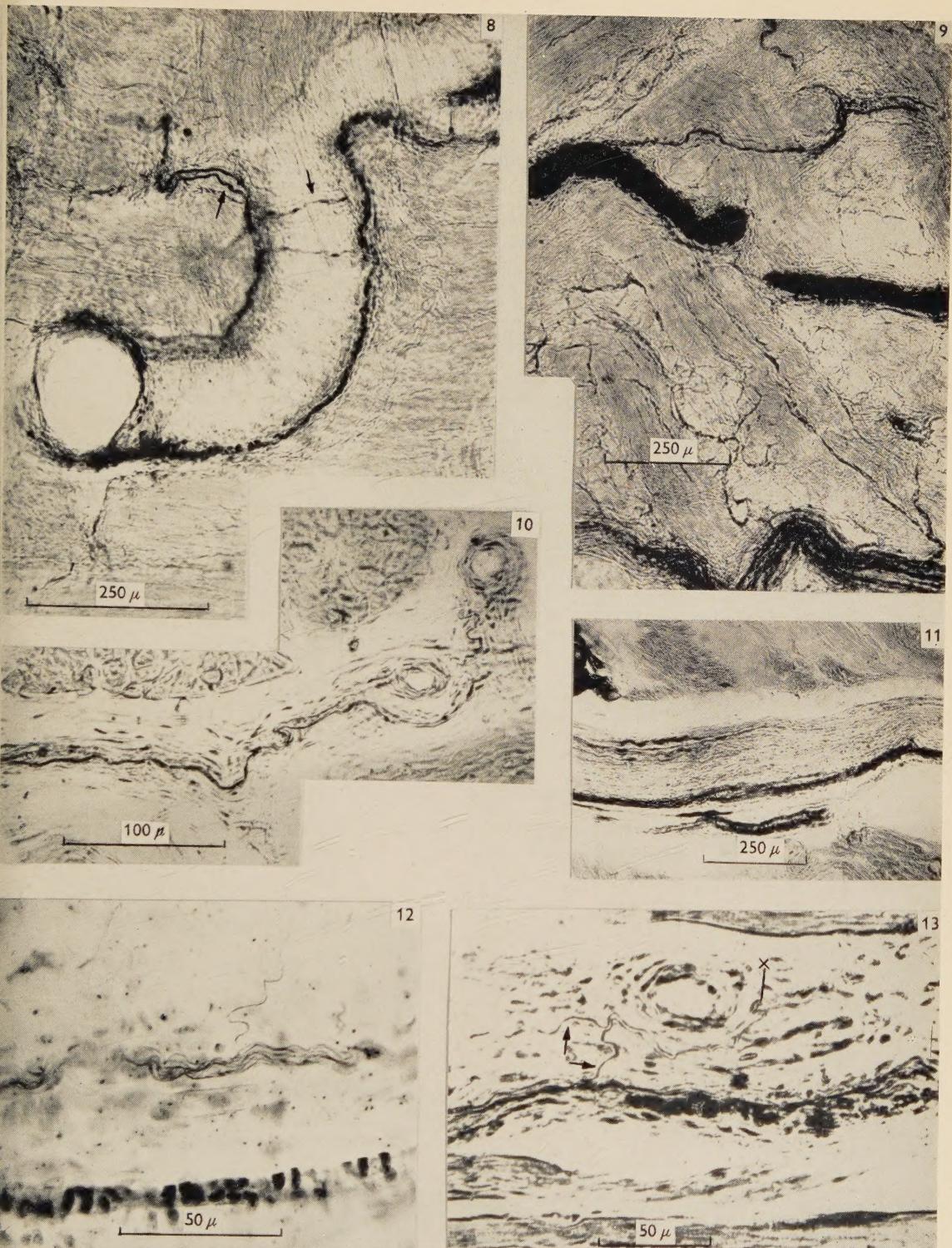


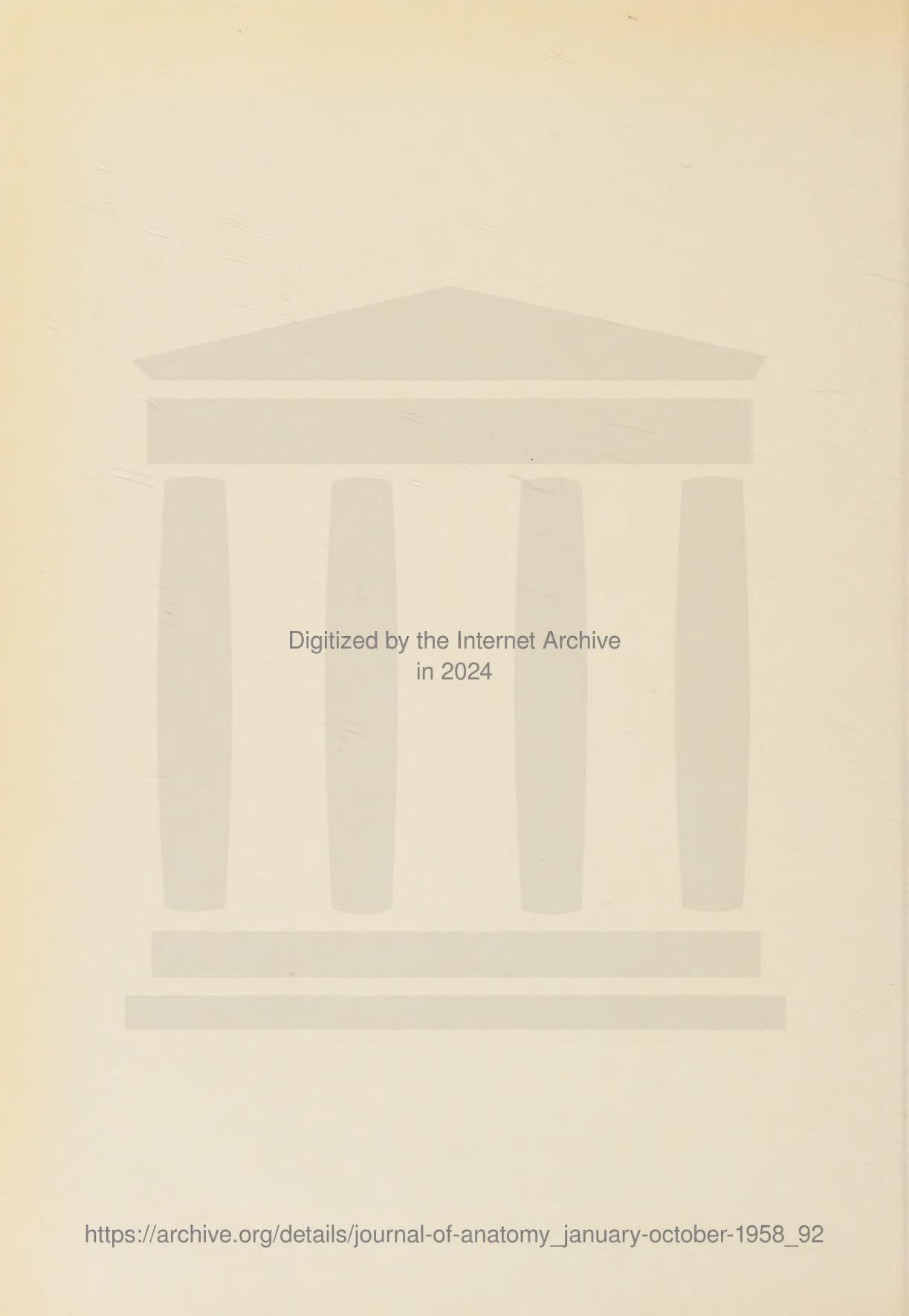


FITZGERALD AND LAW—PERIPHERAL CONNEXIONS BETWEEN LINGUAL AND HYPOGLOSSAL NERVES

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ON THE PRESENCE OF CENTRIPETAL FIBRES IN THE SUPERIOR MESENTERIC NERVES OF THE RABBIT

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On the basis of certain histological and physiological investigations it has been suggested that some of the fibres in the nerve bundles running in the intestinal mesenteries of the rabbit and the cat are centripetal in direction, having their parent cells somewhere in the intestinal wall, and forming synapses in the local mesenteric sympathetic ganglia. Kuntz & Saccomanno (1944) noted that, after sectioning of neurovascular bundles supplying the small and large intestines in the cat, some fibres remained intact in the nerves distal to the interruption. Kuntz (1938) also observed that there was some evidence of degeneration in the proximal segments of those nerves. In addition, he carried out histological studies on the coeliac ganglion (1938) and the inferior mesenteric ganglion (1940) of the cat, and demonstrated that some terminal arborizations in the ganglia remained after interruption of their central connexions.

More recently Brown & Pascoe (1952) have presented physiological evidence of reflex activity in the local mesenteric sympathetic ganglia in the rabbit. The inferior mesenteric ganglion with the ascending mesenteric nerve attached to its cranial pole was removed from the rabbit and placed in Locke's solution in a recording bath. They found that stimulation of the ascending mesenteric nerve produced an impulse that travelled to the inferior mesenteric ganglion. This was followed after a short interval by an outgoing impulse along the nerve. The latter was abolished by painting the ganglion with nicotine and by adding *n*-tubocurarine chloride to the bathing solution. The ingoing impulse was conducted at a rate of 0·25 m./sec. at 20° C., the outgoing one at 0·45 m./sec. These results suggest the presence of fibres ending in the ganglion, and originating in some region peripheral to the ganglion.

Also in 1952, Job & Lundberg obtained similar results in the inferior mesenteric ganglion of the cat by stimulating the hypogastric nerve after complete degeneration of the preganglionic spinal roots of the ganglion.

It is not known where those fibres proceeding to the inferior mesenteric ganglion via the ascending mesenteric nerve have their cell bodies. It has been suggested by McLennan & Pascoe (1954) that perhaps they are situated in the walls of the colon.

It is the purpose of the present investigation to discover if there are centripetally running fibres in the nerve bundles of the small gut mesentery in the rabbit, having their cell bodies situated in the periphery, and if there are, what percentage of the total fibre count they form. To establish this some of the neurovascular bundles in the mesentery of rabbits have been divided between ligatures and the animals allowed to survive for a period of 3 weeks, so that nerve fibres disconnected from their cell bodies degenerate fully. The neurovascular bundle distal to the transection was then removed and examined histologically for surviving nerve fibres.

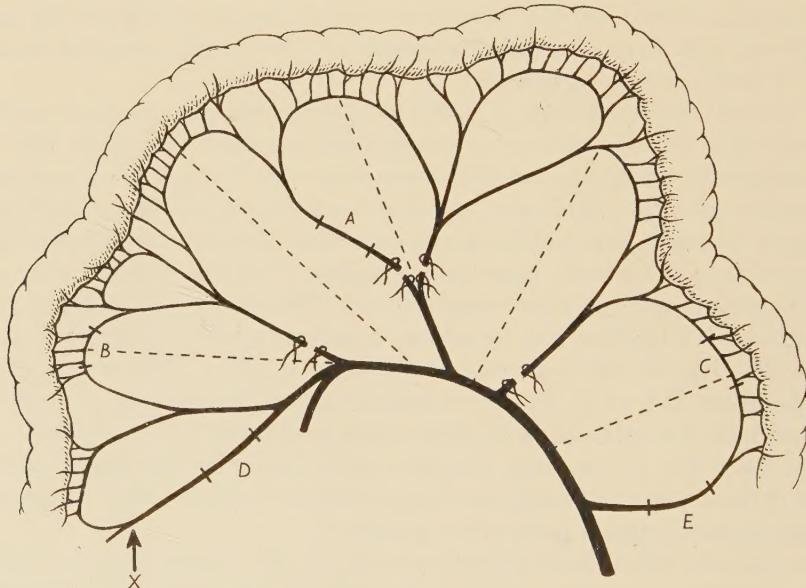
METHODS

Experimental procedure

The operations were performed on adult rabbits of various breeds. The anaesthesia employed was intravenous sodium pentobarbitone (nembutal) supplemented with ether.

Operation

In the first series of animals one to four adjacent neurovascular bundles in the small gut mesentery were doubly ligated near to the main trunk of the superior mesenteric artery, and divided between the ligatures. The cut ends of the bundles were tied together loosely in six animals to prevent excessive retraction, and to help in identification at autopsy. In four animals (4521, 4522, 4537 and 4539) this step was omitted. In addition, the mesentery on either side of each bundle was slit in its entire depth, leaving intact the distal loops of communication between adjacent main branches (Text-fig. 1). By this means a section of mesentery and its contained blood vessels and nerves was 'isolated' except for the communicating loops on either side (B and C in Text-fig. 1) and the length of small intestine supplied by the divided vessels.



Text-fig. 1. Key: A, distal segments; B, C, marginal segments; D, E, control segments; X, site of division of neurovascular bundles in second experiment. ---, slit in mesentery; —, neurovascular bundles.

In two animals (4384 and 4393) the main vagal trunks were divided at the cardia in addition to the above procedure.

When four adjacent branches were interrupted, the affected length of small intestine became slightly cyanotic. Division of less than four branches produced no such change.

The post-operative course in nine of the animals was smooth, and they showed

no apparent intestinal upset. Rabbit 4497 made a normal immediate recovery, but from the fifteenth day would not eat. Autopsy was performed on the sixteenth day, and it was then found that the segment of small intestine whose neurovascular bundles had been interrupted was markedly constricted and white. Elsewhere the intestine appeared normal macroscopically. It was not examined histologically. In this animal only two adjacent branches were divided.

The remaining rabbits were allowed to survive for 21 days to allow complete degeneration of those nerve fibres isolated from their cell bodies.

In a second series of animals three adjacent neurovascular bundles were divided between ligatures placed distally (X in Text-fig. 1). The ligatures were placed proximal to the distal loop of communication to ensure an adequate blood supply to the intestine. The mesentery on either side of the bundles was slit as in the first series. The cut ends of the bundles were tied loosely together.

These animals were left for 21 days before autopsy was carried out.

Autopsy

Fairly dense adhesions were present in the mesentery of nearly every animal, but the neurovascular bundles were dissected clear and identified without difficulty. Where the cut ends of the bundles had been tied together there was a bridge of adhesions between them. In those that had been left free, one or more distal lengths had retracted up to the intestine, and were altogether free of adhesions.

First series of rabbits. Short lengths of the neurovascular bundles distal to the division (A in Text-fig. 1) were excised, threaded through spinal cord previously obtained from the rabbit, and retained in a slightly stretched condition on a cardboard frame. Similar lengths were taken from intact neurovascular bundles and from the communicating loop on either side of the operation field (B and C in Text-fig. 1), and treated in the same manner.

The distal segments of mesentery that had retracted in rabbits 4521, 4522, 4537 and 4539 were selected for biopsy as it was possible that regenerating nerve fibres might have bridged the gap between the tied ends of the divided bundles. Some of these adhesions have been examined and none of them showed continuity of nerve fibres between the proximal and distal segments.

Second series of rabbits. Short lengths of bundles proximal to the division were excised, and treated similarly.

Staining

The bundles, together with the spinal cord were fixed in alcohol ammonia solution, stained by Ranson's pyridine silver method (Ranson & Davenport, 1931) and at the completion of the staining process both the cord and its contained bundle were embedded in paraffin and sectioned transversely as near $1\text{--}2\mu$ thickness as was possible. It was found that in sections thicker than $3\text{--}4\mu$ the nerve fibres were difficult to count accurately.

RESULTS

Histological findings

Full counts were carried out on all the specimens. Most of them contained a number of nerve bundles—varying from one to nine. The results of these counts are shown in Table 1 (first series) and Table 3 (second series).

The number of nerve fibres per unit area was also calculated by the following method:

Each bundle counted was projected on to a ground glass screen at a magnification of 1000, and traced on to tracing paper. The tracings were then cut out and weighed on a microbalance. Unit weight was taken to be the weight of 1 cm.² of tracing paper. The results of these calculations are also shown in Tables 1 and 3.

Table 1. *Total counts and counts per unit area in first series of rabbits*

Rabbit	Operation	Normals		Distal segments		Marginals	
		Total no.	Per unit area	Total no.	Per unit area	Total no.	Per unit area
4348	Four adjacent branches divided proximally	287*	155	297	61	134	91
4384	One branch divided proximally + division of vagi at cardia	532*	222	99	45	116	92
4393	One branch divided proximally + division of vagi at cardia	1421	132	199	72	295	46
4394	Two adjacent branches divided proximally	1459	154	610	52	231	27
4497	Two adjacent branches divided proximally	1337	221	349	39	78	89
4521	Four adjacent branches divided proximally	1338	169	704	67	469	55
4522	Four adjacent branches divided proximally	1599	291	138	69	30	228
4530	Four adjacent branches divided proximally	1063	148	543	47	63	217
4537	Four adjacent branches divided proximally	2264	145	744	92	48	143
4539	Four adjacent branches divided proximally	4144	161	613	87	76	217

Normals: mean per unit area 174, standard deviation of mean 22.

Distal segments: mean per unit area 63, standard deviation of mean 6.

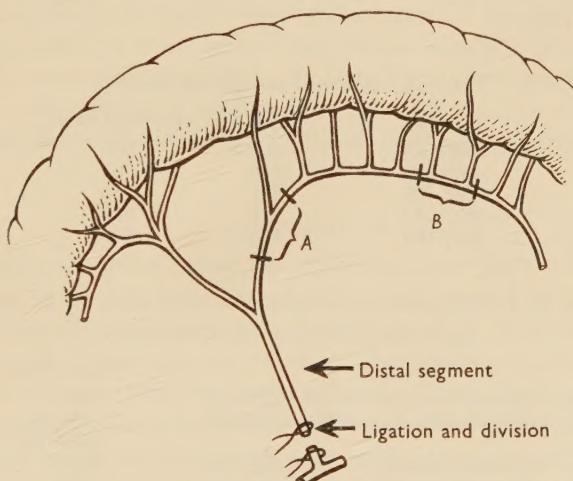
* These normal segments were taken distal to the division of a main branch of the superior mesenteric artery.

A study of Table 1 indicates a wide variation in the number of fibres in the control specimens, but the counts per unit area are more constant—132–291 with a mean of 174 (standard deviation of mean 22). It is interesting to note that in the normal nerve bundles, the individual nerve fibres are not very closely packed together. Some of the variation in this respect may be due to shrinkage or oedema of the specimens produced during the fixation and staining process. One or two of the nerve bundles examined show a clear zone round the periphery where the fibres had retracted away from the perineurium. In these cases the tracing followed the perineurium.

In making counts some difficulty was encountered, particularly in the control and marginal specimens where two or more nerve fibres were very close to one another. It was not always easy to tell whether there were two to three fibres present, or whether one was looking at a large single deposit of silver. This difficulty was obviated largely by using sufficiently thin sections, and it was found essential for accuracy to use only sections cut at less than 3–4 μ for counting purposes. It is not easy to prepare good sections at this thickness, and many of the nerve bundles tended to break up. Where this had occurred, closely adjacent thicker sections were

used for the calculations of the area. Occasionally one or more nerve bundles were cut obliquely. Usually recutting in a different plane corrected this error.

The total counts in the distal segments range from 99 to 744, and the counts per unit area from 39 to 92 with a mean of 63 (standard deviation of mean 6). In other words, the remaining intact fibres represent approximately 30 % of the total present in the control segments. These fibres are not evenly scattered through the individual bundles. Some of the latter contain many fibres and are apparently little different from the controls. On the whole, however, the decrease in density is obvious on examination. The majority of the intact fibres are of small calibre—probably 1μ or less in diameter.



Text-fig. 2. Diagram to give possible explanation of varying counts per unit area in marginal bundles.

It is possible that some of those fibres remaining in the distal segments could be accounted for by nerve fibres entering from either side through the marginal nerves. The total counts of the marginal bundles are therefore of more significance than the counts per unit area. Table 1 shows that in only two animals (4384 and 4393) do the nerve fibres in the two marginal bundles outnumber those remaining in the distal segment. In all other rabbits it is clear that they cannot account for all the nerve fibres remaining unless one postulates branching of the axons in the marginal bundles.

Both the total counts and the count per unit area vary much more in the marginal nerves than they do in the control and distal segments. Some of the bundles have the appearance of normal nerves, and others of distal segments, and it seems that the appearance is dependent upon the exact site chosen for biopsy. When the segment lies close to the operation field (A in Text-fig. 2) the nerves are similar to the distal segments. Further away (B in Text-fig. 2) they look like the controls.

As far as was possible the segments of the marginal neurovascular bundles chosen for study were equidistant from the adjacent normal and divided neurovascular bundles (Text-fig. 2).

It seems, therefore, that the majority of the nerve fibres running in the marginal bundles travel with the short terminal branches of the vessels into the intestine, and do not retrace their path to pass centrally in an adjacent main neurovascular bundle. Presumably some of these fibres remaining in the marginal nerves that have the appearance of the distal segments are similar fibres to the intact ones in the distal segments. The counts per unit area of the marginal nerves are probably not accurate, as most of the bundles are very small.

Table 2 shows the total counts and the counts per unit area of normal marginal bundles.

The results obtained in the second series of rabbits are shown in Table 3. The counts per unit area vary between 68 and 156, with a mean of 100 (standard deviation of mean 8).

Table 2. *Normal marginal bundles*

Rabbit	Total No.	Per unit area
4825(A)	420	82
4825(B)	452	81
4825(C)	251	88
4831(A)	117	135
4831(B)	108	208

Table 3. *Total counts and counts per unit area in proximal segments following distal division*

Rabbit	Operation	Total count	Per unit area
4760	Three adjacent branches divided distally	1545	78
4761	Three adjacent branches divided distally	1538(A)	93
		1459(B)	95
4829	Three adjacent branches divided distally	2638	156
4843	Three adjacent branches divided distally	1502	113
4844	Three adjacent branches divided distally	2223(A)	94
		1402(B)	105
4858	Three adjacent branches divided distally	2044(A)	84
		1532(B)	119
4863	Three adjacent branches divided distally	2579	68

Mean per unit area 100, standard deviation of mean 8.

Confirmation of the quantitative changes in fibre count using the second series of rabbits is not ideal as the difference to be expected is only in the region of 30 %, and the experimental error is probably greater than 10 %. Some individual counts per unit area in the distal segments of the first series, and in the specimen of the second series of animals show an overlap, although the mean figures per unit area are of significant difference.

An attempt was made to demonstrate degeneration in the proximal segment following distal divisions of the neurovascular bundles. Various silver stain techniques were used on specimens taken from 1 to 15 days following operation. In none of these was there unequivocal evidence of degeneration. In order to test the efficiency of the methods employed distal segments were studied after either proximal division or excision of the superior ganglion on the left side. Again, no firm evidence of degeneration was obtained, and it appears that the techniques used were not capable of staining the degeneration granules of such fine nerve fibres. Certainly

the fibres remaining in the distal segment after proximal division are unmyelinated and of small diameter.

Using Nauta's silver stain (1954), degeneration granules of the preganglionic fibres in the greater splanchnic nerves and of the preganglionic supply to the superior cervical ganglion have been shown, but an attempt to show degeneration in post-ganglionic fibres using this stain has failed.

In a third series of rabbits the more peripheral parts of the mesentery were examined for outlying ganglion cells. Two rabbits were used (4877 and 4878), and in each rabbit six lengths of neurovascular bundles were excised from the marginal loops, and six from the proximal ends of the main neurovascular bundles. Each bundle was held in a cardboard frame in a slightly stretched condition, and was fixed in Bouin's solution for 48 hr. The specimens were then dehydrated, cleared, embedded in wax, and 5μ thick serial longitudinal sections cut. The sections were stained with haematoxylin and eosin on the slides. One ganglion cell was discovered in one of the proximal bundles in rabbit 4877, and possibly one in a distal segment. No ganglion cells were seen in the nerve bundles removed from the second rabbit.

DISCUSSION

The intact fibres in the distal segments apparently have their cell bodies in the wall of the gut. There is an alternative possibility that some of these fibres arise from ganglion cells situated in the mesentery distal to the cut. Recently Kuntz & Jacob (1955) have carried out an investigation on the periarterial extension of coeliac and mesenteric plexuses in the rat, cat and human. They found some ganglion cells along the mesenteric arteries and their main branches. The present investigation shows clearly that nearly all these fibres arise within the gut itself and not in ganglia cells in the peripheral part of the mesentery. It remains to be discovered whether they are processes of cells in the intrinsic plexuses.

The termination of the fine centripetal fibres is not known. It is possible that they form part of a local reflex mechanism with synaptic connexions in the superior mesenteric ganglion, and Kuntz (1938) has presented some anatomical evidence that this is the case. If they do so, such a local reflex mechanism must be of great importance as these fibres constitute no less than 30 % of the total number of nerve fibres running in the branches of the superior mesenteric nerve.

As already outlined, there is physiological evidence that reflexes can occur through the decentralized abdominal sympathetic ganglia. Kuntz & Saccoccanno (1944) carried out both acute and chronic experiments on the inferior mesenteric ganglion of the cat. In the acute experiments the central preganglionic inflow into the ganglion was interrupted by extirpating the spinal cord below the level of the cervical cord, and dividing the vagi at the cardia. In the chronic experiments, they divided the lumbar sympathetic trunk bilaterally, the hypogastric nerves and the coeliac roots, and allowed an interval of 7 days before proceeding with the experiment. In both the acute and chronic experiments they found that the intestino-intestinal reflex persisted, i.e. after the large gut was divided leaving its two segments connected only by mesentery, inhibition of the proximal segment occurred on raising the intra-luminal pressure in the distal segment. They also observed that faradic

stimulation of the nerves supplying the distal segment of intestine produced inhibition of the proximal segment of gut. They carried out similar experiments on the small intestine.

It is difficult to be certain in all cases that the central preganglionic inflow into the mesenteric ganglia is completely interrupted, and it would be additional evidence of local reflexes through the ganglia if the intestinal response was abolished by subsequent removal of the ganglia.

It cannot be assumed that the nerve fibres described in this paper are similar to those described by Brown & Pascoe (1952) in the ascending mesenteric nerve, but the likelihood is that they are. The peripheral preganglionic fibres described by these authors were of small diameter judged by their velocity of conduction (0.25 m/sec.), and they apparently had their cell bodies somewhere in the peripheral distribution of the ascending nerve, and probably in the walls of the colon. McLennan & Pascoe (1954) attempted to determine the origin of these fibres, and they demonstrated that they remained intact after chronic decentralization of the inferior mesenteric ganglion, bilateral splanchnicotomy, bilateral vagotomy, and bilateral abdominal sympathectomy. Following solar ganglionectomy degeneration of some of them may occur.

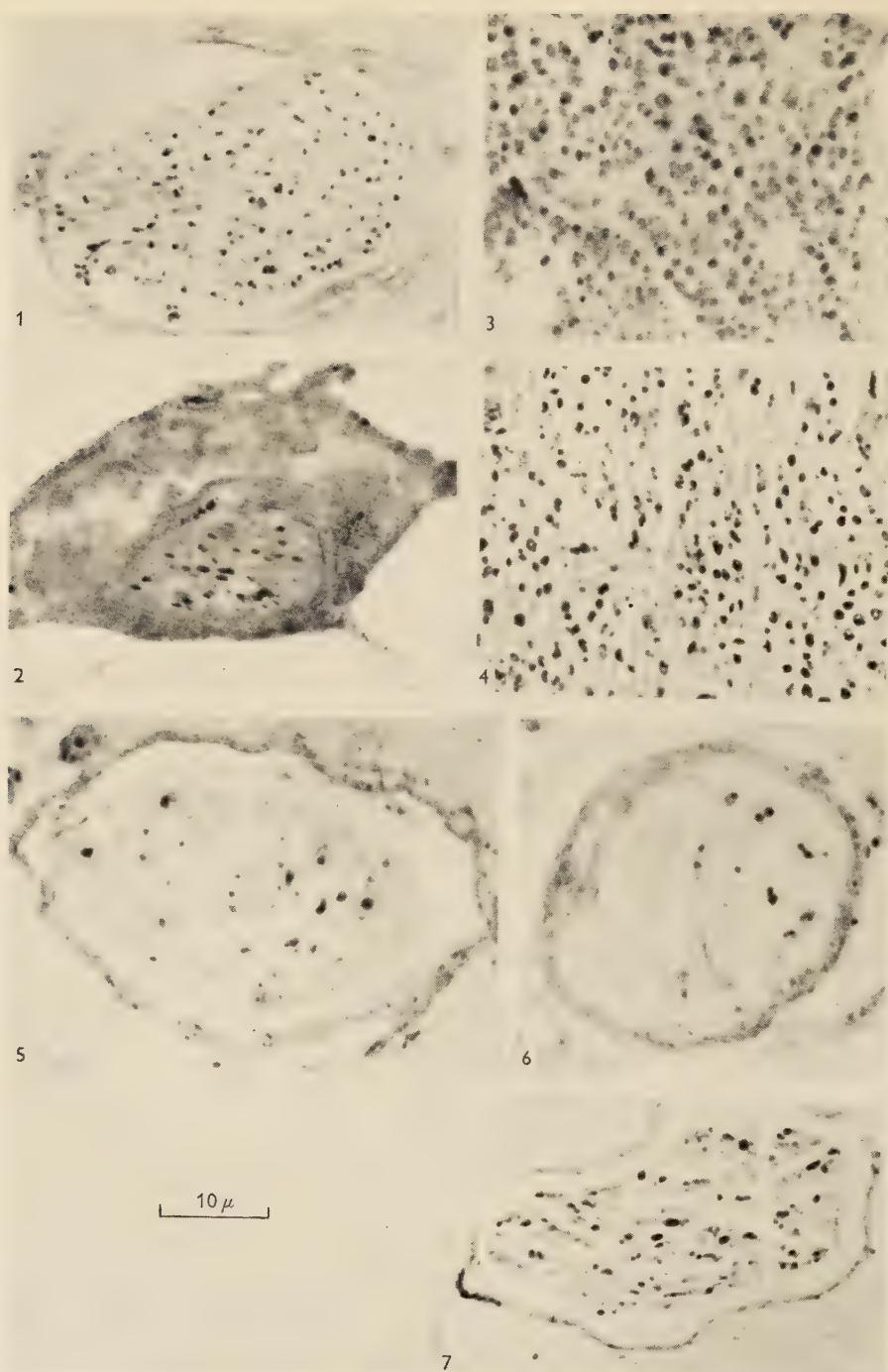
In the present series, bilateral vagotomy was carried out on two of the rabbits. Although this is not sufficient to base any firm views on, the likelihood of the nerve fibres described being of vagal origin is remote, and in these two animals, no significant difference in total counts or counts per unit area was discovered.

It would appear that those nerve fibres with their cell bodies situated apparently in the intestine are similar to those described by Kuntz (1940) in the colonic neurovascular bundles. Their function is not known, but it is suggested that perhaps they are of importance in the intersegmental activity of the intestines.

SUMMARY

1. Following division of neurovascular bundles in the small gut mesentery of the rabbit, and allowing a period of 21 days for degeneration to occur, some nerve fibres remain intact distal to the interruption.
2. Counts per unit area show that these fibres represent 30 % of the fibres present in the normal superior mesenteric neurovascular bundles.
3. It is suggested that these fibres have their cell bodies situated in the gut wall, and that they are proceeding centripetally to the local sympathetic ganglia to effect synapse there.
4. Physiological evidence of local reflex activity through these ganglia is discussed.

I wish to thank Prof. J. Z. Young, and Dr D. H. L. Evans for their helpful criticism and advice, Miss Pamela Hines for her technical assistance, and Mr J. Armstrong for taking the photographs.



ROSS—CENTRIPETAL FIBRES IN SUPERIOR MESENTERIC NERVES OF RABBIT

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EXPLANATION OF PLATE

Figs. 1 and 2. Transverse sections of distal segments of nerves following division of superior mesenteric neurovascular bundles proximally. The nerve fibres remaining are the centripetal ones referred to in the text.

Fig. 3. Transverse sections of a normal nerve bundle in the mesentery of the small intestine. The nerve fibres are not closely packed.

Fig. 4. Transverse section of a proximal segment of nerve bundle following division of a superior mesenteric neurovascular bundle distally. The fine centripetal nerve fibres have degenerated.

Figs. 5 and 6. Transverse sections of marginal nerve bundles. The first section contains more nerve fibres per unit area than the second, and is more comparable with a normal marginal bundle. It was taken from a marginal loop near to an intact main neurovascular bundle. The second one was taken close to a divided main neurovascular bundle.

Fig. 7. A normal marginal nerve bundle.

**AN ANATOMICAL ANALYSIS OF CORTICO-BULBAR
CONNEXIONS TO THE PONS AND LOWER BRAIN
STEM IN THE CAT***

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I. INTRODUCTION

Ever since the pyramidal tract was recognized by Gall & Spurzheim (1810) as a decussating fibre bundle from the cerebral cortex to the spinal cord, much attention has been paid to this fibre system. Nevertheless, there still exists considerable uncertainty concerning the pathways along which the corticofugal connexions of the pyramidal tract to the pons and the medulla oblongata reach the motor nuclei of the cranial nerves. Clinical observations have led to certain inferences concerning such cortico-bulbar pathways, for example, an apparent difference in cortical projections to different parts of the facial nucleus. In spite of a considerable amount of morphological work, the anatomical explanation of such clinical phenomena remains obscure. This is largely the result of restrictions inherent in the available techniques. The experimental anatomical analysis of the pyramidal tract connections, as predominantly performed with the classical Marchi method, is by its very nature often unable to demonstrate the exact sites of termination of degenerating fibres. On the other hand, due to the lack of adequate human material, most of the revelant information has been obtained from experiments in lower animals, though these have included the monkey.

Some of the limitations imposed by the Marchi method were avoided in the present study by the use of the selective silver impregnation of degenerating nerve fibres following Nauta & Gygax (1954), which allows satisfactory identification of degenerating axons including their end-ramifications.

In regard to the paucity of suitable human material, the information obtained by the present experiments in the cat was expected to facilitate the study of several human specimens which is completed now and, together with observations in monkey and chimpanzee material, will be reported separately at a later date.

* The early part of this work was carried on at the Department of Neurology, University of Groningen, Netherlands. This study together with the first results of the investigations in man were presented at the meeting of the American Association of Anatomists, Milwaukee, Wisconsin, U.S.A., on 4 April 1956. This investigation was supported in part by a research grant no. B. 1131 from the Division of Neurological Diseases and Blindness of the U.S. Public Health Service.

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II. MATERIALS AND METHODS

The material consisted of fourteen cat brains containing various surgical lesions, in which the distribution of the degenerating nerve fibres was studied. The experimental material (Text-fig. 1) can be categorized as follows:

1. Practically total unilateral hemispherectomy with involvement of the basal ganglia (2 cases: C₁ and C_{1a}).
2. Removal of practically the entire surface of one hemisphere without involvement of the basal ganglia (2 cases: C₂ and C₃).
3. Extensive lesion of the white matter of the rostral part of one hemisphere, including that of the motor cortex as outlined for the cat by Garol (1942) (1 case: C₄).
4. Partial removal of the frontal cortex, including parts of the face and forelimb areas of the motor cortex (1 case: C₅).
5. Removal of part of the face area of the motor cortex (1 case: C₉).



Text-fig. 1. This figure shows location and extent of the lesions in the material studied, superposed upon the functional map of the cat's motor cortex as analysed by Garol (1942).

6. Removal of part of the forelimb area of the motor cortex (1 case: C₆).
7. Removal of a part of the hindlimb area of the motor cortex (2 cases: C₁₂, C₁₃).
8. Removal of part of the hindlimb and an adjacent part of the forelimb area (2 cases: C₁₀, C₁₁).
9. Removal of the most medial part of the anterior sigmoid gyrus at the dorsal aspect of the hemisphere (1 case: C₈).
10. Removal of the most medial part of anterior sigmoid gyrus and an adjacent part of the forelimb area (1 case: C₇).

The lesions were produced by superficial aspiration of cortex. The postoperative survival period varied between 7 and 12 days. Subsequently, the animal was anaesthetized and perfused with isotonic saline followed by 10% neutral formalin. The brain was then removed and stored in 10% neutral formalin for at least 14 days. Serial frozen sections of 25 micra thickness, cut in the sagittal or the transverse plane, were stained by the technique of Nauta & Gygax (1954).

III. RESULTS

(a) Experiments of the categories 1, 2, 3 with brain stems cut in transverse sections

To facilitate the interpretation of subsequent observations the experiments with hemispherectomy, extensive cortical damage, and extensive white-matter lesion of the rostral part of the hemisphere will be described first. The results from all three types of lesions were strikingly similar and can be described collectively.

At the upper levels of the cervical spinal cord (Text-fig. 2: 1) the area of the lateral cortico-spinal tract contralateral to the lesion was filled with degenerating fibres. Only a few such fibres were found in the same region on the opposite side. The ipsilateral anterior funiculus also contained a small number of degenerating fibres. Fibres from the lateral cortico-spinal tract were found to enter the grey substance of the spinal cord and could be traced to cells situated in the nucleus proprius, the basal parts of the dorsal horn, and the zona intermedia, with the highest concentration in the basal parts of the dorsal horn. The same region of the opposite side contained only a few degenerating fibres. In the dorsal region of the grey substance, close to the border with the dorsal funiculus, an accumulation of degenerating longitudinal fibres was noticed. Extremely few degenerating fibres and end-ramifications could be found in the ventral horn.

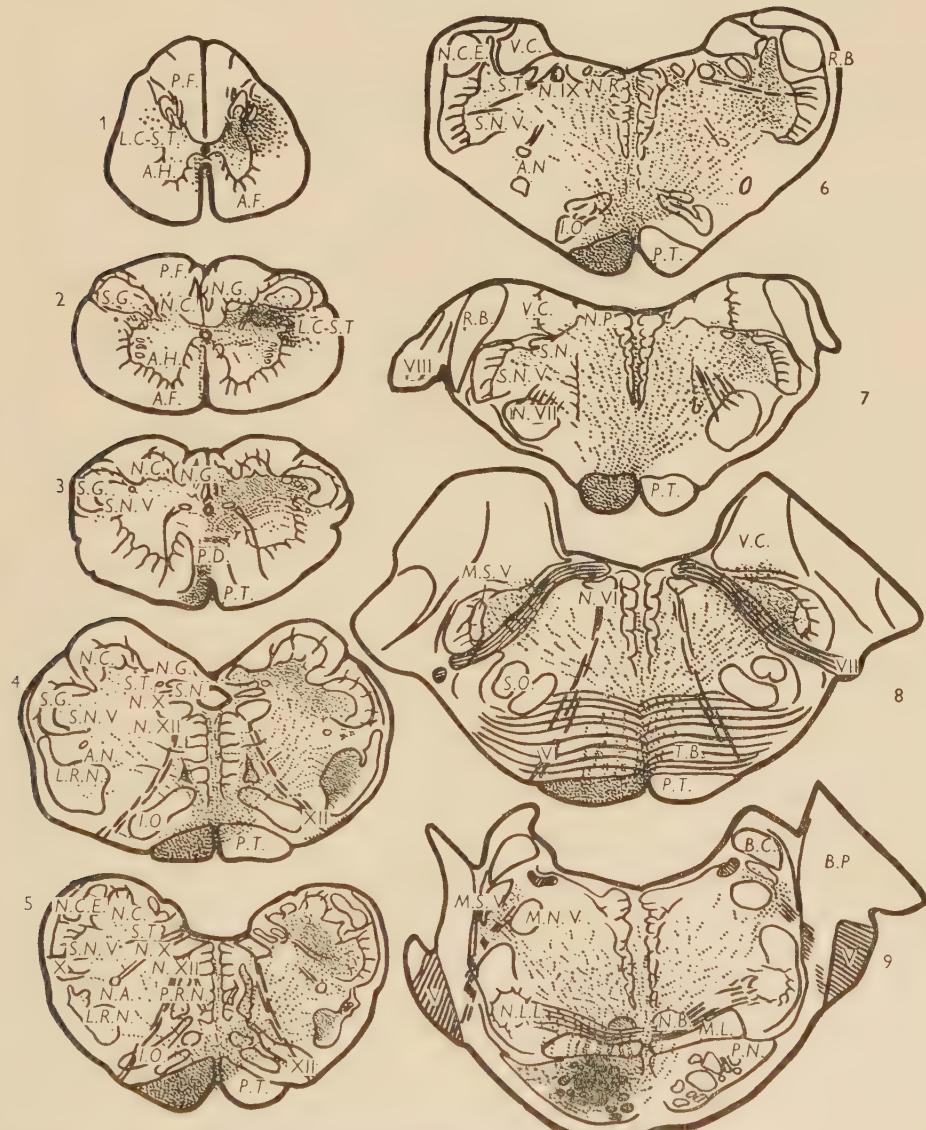
At slightly higher levels (Text-fig. 2: 2) the same distribution of degenerating axons was observed. The nucleus cuneatus appears here as a projection from the medio-dorsal edge of the dorsal horn into the dorsal funiculus, and is thus located just dorsal to the region of degeneration.

More rostrally the decussation of the pyramidal tracts is present (Text-fig. 2: 3), through which massive degeneration could be traced into the contralateral cortico-spinal tract, a small number of fibres remaining uncrossed. At these levels degeneration was present in a broad area just ventral to and in the hilus of the nuclei cuneatus and gracilis, extending from the midline into the spinal trigeminal nucleus. Especially in the nucleus gracilis the degeneration extended deeply into the hilus of the nucleus. At some higher levels the degeneration appears even to involve the nucleus gracilis proper. This accumulation of degenerating fibres represented a rostral continuation of that observed in the corresponding region of the spinal cord. At certain levels degenerating fibres could be traced to this region in continuity through the pyramidal decussation. Very few, if any, degenerating end-ramifications appeared among the cells of the hypoglossal and accessory nuclei. A diffuse scattering of degenerating fibres was also present throughout the region ventral to the transverse trajectory of the pyramidal bundles. All of the degenerating fibres described above were present on both sides, but those contralateral to the lesion were more numerous.

To obviate the separate description of a great many medullary levels, the medulla oblongata will be described as a unit divided into three parts: the caudal region containing the main olives, the intermediate region containing the facial nucleus and the rostral region characterized by the trapezoid body.

The pyramidal tract ipsilateral to the lesion was found to release degenerating fibres in the dorsal direction throughout its entire course. Such dorsal offsets were most numerous in the intermediate and adjacent part of the caudal region. In the

caudal and intermediate regions, degenerating fibres were seen to spray out from between both tracts in a fountain-like fashion (Text-fig. 2: 5) and in addition left the pyramidal tract through its dorsolateral and through its medial sides. These fibres distributed partially into the ipsilateral and partially into the contralateral half of the medulla oblongata. In the rostral region comparable fibre groups left the tract predominantly at the medial corner. In the caudal region the fibres of both groups



Text-fig. 2. Semi-diagrammatic representation of axon degeneration distributing to the pons, medulla oblongata and cervical spinal cord in transverse sections, following extensive lesion to the frontal lobe as illustrated in Text-fig. 1 (C₄). Coarse stipple indicates degenerating fibres of passage; fine stipple, degeneration of terminal ramifications. Abbreviations, see p. 218.

coursed through the dorso-medial part of the olfactory complex to reach the tegmentum. A few of these fibres may terminate in the olives, but due to the many fibres of passage the amount of degenerating terminals was difficult to estimate. The corticofugal fibres in the caudal region largely passed into the contralateral half of the brain stem, but in the intermediate and rostral regions the distribution appeared to become more bilateral, with a predominance of contralateral projections.

These fibres of the pyramidal tract distributed diffusely throughout the tegmentum. However, local differences in the density of their termination appeared as follows.

In the caudal region of the medulla (Text-fig. 2: 4, 5) the areas of greatest density were: the magnocellular part of the contralateral lateral reticular nucleus as well as the medial part of the contralateral spinal trigeminal nucleus together with adjoining parts of the dorso-lateral tegmentum, and the region immediately ventral to, and in the hilus of, the nuclei cuneatus and gracilis.* The bulk of the magnocellular lateral reticular nucleus received a large number of degenerating fibres. In its most rostral parts, the magnocellular part of the nucleus is divided into a medial and a lateral cell group. The former received the same large number of fibres as the main body of the nucleus but the medial cell group showed fewer signs of terminal degeneration. However, this part of the lateral reticular nucleus is locally very difficult to delimit from the adjoining reticular formation. Accumulation of degenerating elements just ventral to and in the hilus of the nuclei cuneatus and gracilis was found to accompany these nuclei along their entire extent in the brain stem. The corresponding areas in the other half of the brain stem contained only a few degenerating fibres.

Nuclei which appeared to receive lesser numbers of degenerating fibres were the paramedian reticular nuclei of both sides and part of both nuclei of Roller. In respect to the latter, end-ramifications were observed in medial and ventral parts of the nucleus, which bend ventrally around the anterior pole of the hypoglossus nucleus.

In analogy to the findings in the cervical spinal cord, the motor nuclei of the cranial nerves contained few if any degenerating fibres.

At the level of the rostral part of the main olive and in the intermediate region of the medulla (Text-fig. 2: 6, 7) the number of corticofugal fibres was larger than at a more caudal level and exhibited only a slight preference for the contralateral half. They coursed in two main directions: dorso-medial and lateral. The dorso-medially directed fibres distributed over the central part of the tegmentum, which appeared to represent an area of preferential distribution. The laterally directed fibres distributed over the lateral part of the tegmentum and could in part be traced into the spinal trigeminal nucleus, after passing the facial nucleus, which itself appeared to receive few if any such fibres. Contrary to the findings at more caudal levels, the degenerating fibres and end-ramifications were clearly present in both the left and

* The spinal trigeminal nucleus is difficult to delineate from the parvicellular lateral parts of the reticular formation, which adjoins it on the medial side. Nevertheless the present findings leave little doubt that the cortical projections here described actually distribute to cell groups, which form part of the spinal trigeminal nucleus, as well as to the adjoining parts of the dorso-lateral tegmentum.

the right spinal trigeminal nucleus, although in larger numbers in the one contralateral to the lesion (Text-fig. 2: 7).

In the most rostral parts of the medulla oblongata, containing the trapezoid body, corticofugal fibres were less numerous, but the general pattern of distribution was the same (Text-fig. 2: 8). Again, degenerating fibres fanned out through the entire tegmentum and two main groups could be distinguished passing to the medial part and to the lateral part of the tegmentum respectively. Fibres of the lateral group could also in part be traced into the spinal trigeminal nucleus of both sides, by passing the superior olive nuclei.

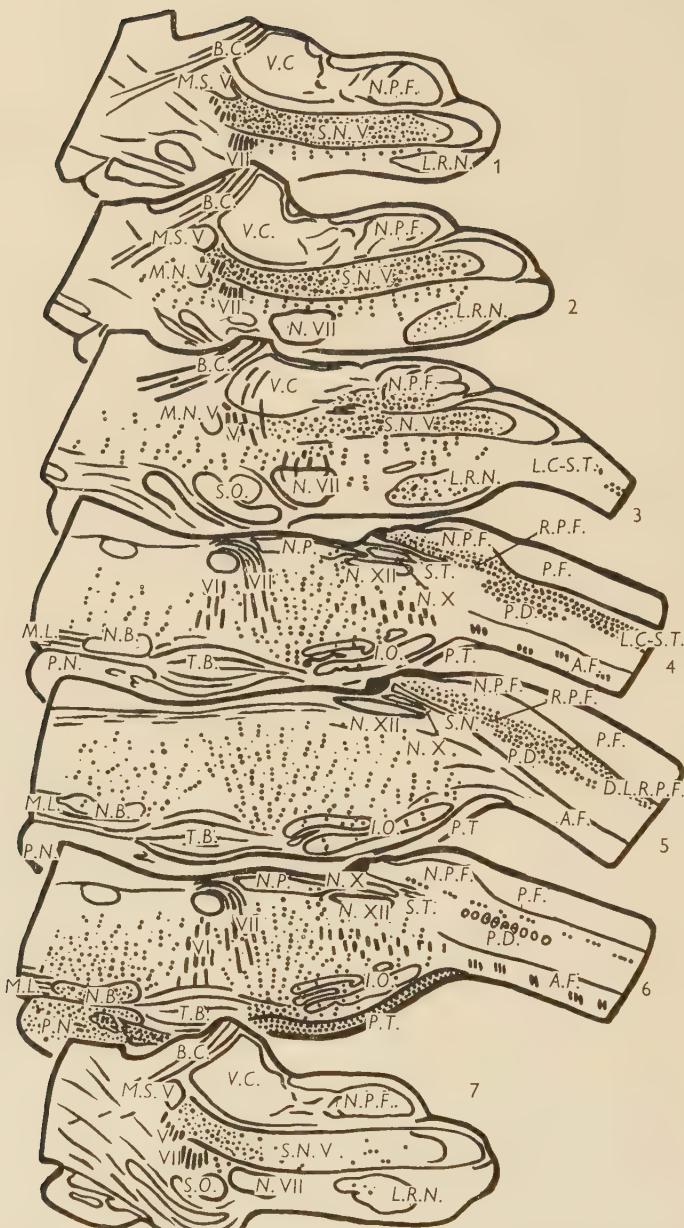
Corresponding to the findings in the caudal and intermediate regions as well as in the spinal cord, few if any degenerating end-ramifications were present in the abducent nuclei.

At the pontine levels (Text-fig. 2: 9) the general internal structure changes considerably. Here the pontine nuclei contained a large number of degenerating elements. From this region degenerating fibres could be traced through the medial lemniscus and the opposite half of the pontine grey into the tegmentum. These fibres terminated in the ipsilateral processus tegmentalis pontis of Bechterew as well as in the more ventral parts of the tegmentum, especially on the ipsilateral side. Some of them could be traced to both trigeminal areas. At these levels, however, the projection to the 'trigeminal region' appeared to be confined to a zone of reticular formation interposed between the main sensory nucleus and the motor root of the trigeminus. It was about equally massive on both sides and did not involve the main sensory nucleus proper. This juxta-trigeminal degeneration continued rostrally a short distance beyond the pontine trigeminal nuclei, into the region adjoining the ventral and medial aspect of the brachium conjunctivum at the level of the isthmus.

In the spinal cord longitudinally directed degenerating fibres were present in a region at the dorso-medial border of the dorsal horn. In addition, a large number of the degenerating fibres at the ventral aspect of the nuclei cuneatus and gracilis exhibited a longitudinal direction. Finally, the number of degenerating fibres which left the pyramidal tract and could be traced through the opposite tegmentum was not sufficiently large enough to explain the great number of degenerating elements present in that region. These observations indicated the necessity of studying the brain stem and spinal cord in sagittal sections.

(b) Experiments of categories 2 and 4 with brain stems cut in sagittal sections

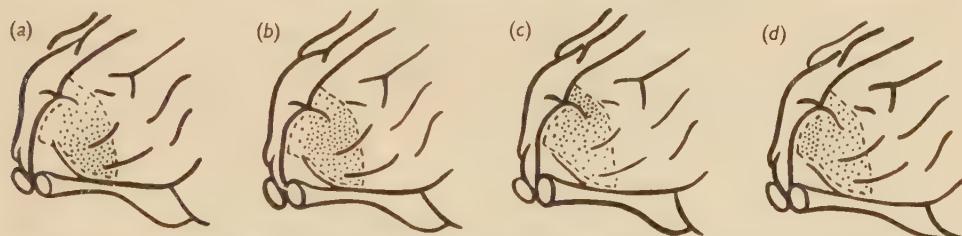
In the region bordering the ventral aspect of the nuclei cuneatus and gracilis a number of degenerating fibres could be observed which appeared to leave the bundles of the pyramidal tract just distal to the decussation, in the dorsal direction (Text-fig. 3: 4, 5; *R.P.F.*). These fibres bent rostralwards as recurrent bundles, and in part also caudalwards. The recurrent fibres occupied a region ventral to the nuclei cuneatus and gracilis accompanying them throughout their caudo-rostral extent. Along their course rostralwards these fibres diminished in number, apparently by distributing to the cells located in that region. The fibres released from the pyramidal tract in the caudal direction could be traced into the dorsal part of the grey substance of the cervical spinal cord (Text-fig. 3: 5, *D.L.R.P.F.*), paralleling the bundles of degenerating lateral cortico-spinal tract. Additional longitudinal fibres were



Text-fig. 3. Semi-diagrammatic representation of the distribution of degenerating fibres to the pons, medulla oblongata and cervical spinal cord, in sagittal sections, following restricted lesion of the sensori-motor cortex (Text-fig. 1, C₅). Symbols as used in Text-fig. 2. Abbreviations, see p. 218.

observed in the medial part of the spinal trigeminal nucleus and its vicinity, especially in its caudal subdivision (Text-fig. 3: 1-3). The above-mentioned recurrent fibres were present contralateral to the lesion and only a few such fibres could be observed in the corresponding region of the ipsilateral side. A comparison of these observations with those in the transverse sections suggested that the group of degenerating fibres running longitudinally in the posterior part of the spinal grey substance represented the descending limb of a system of recurrent pyramidal bundles coursing along the ventral aspect and in the hilus of the nuclei cuneatus and gracilis. Finally, the recurrent fibres furnished a likely explanation for the rather heavy accumulation of degenerating elements at the ventral aspect and in the hilus of the nuclei cuneatus and gracilis, which could not be satisfactorily accounted for by the trans-tegmental corticofugal fibres only.

In addition, the course of the fibres to the tegmentum of the medulla oblongata and the pons could be studied in these sections. Again it became evident that the corticofugal fibres to the medial part of the medulla left the pyramidal tract predominantly in the intermediate region. They streamed from below into a narrow



Text-fig. 4. Diagrammatic approximation of the relative density of origin of various cortico-bulbar projections in the area covered by the experiments: (a) projections to the spinal trigeminal nucleus and its vicinity, especially its rostral part; (b) projections to the region along the ventral aspect and in the hilus of the nucleus cuneatus; (c) projections to the region along the ventral aspect and deeply in the hilus of nucleus gracilis; (d) projections to the central tegmental area of the middle third of the medulla oblongata.

pass between the trapezoid body rostrally and the inferior olive caudally and sprayed out from between both structures into the tegmentum in a fountain-like fashion, covering the intermediate region as well as the adjoining parts of the rostral and caudal regions of the medulla oblongata (Text-fig. 3: 4-6).

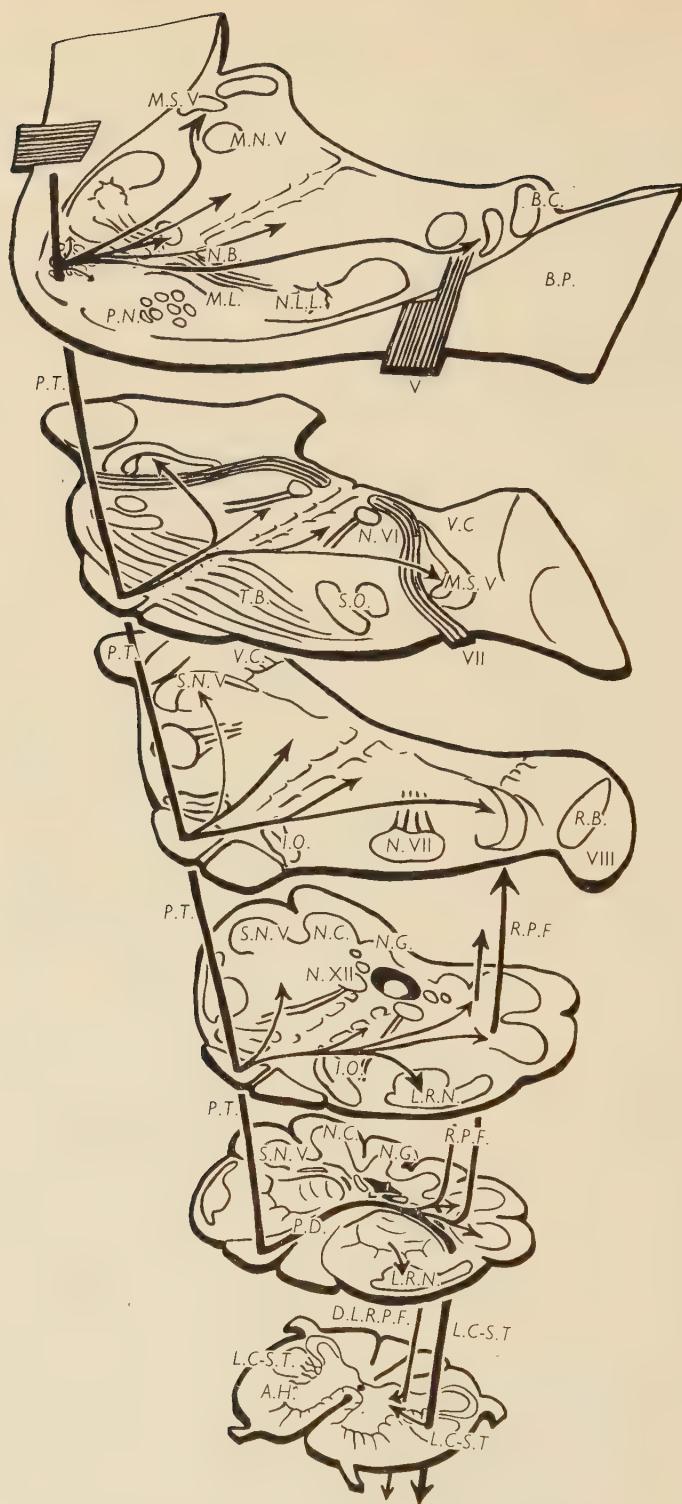
In the rostral region a small number of degenerating fibres could be traced from the pyramidal tract through the trapezoid body into the overlying tegmentum. Similar fibres were observed in the caudal region, coursing through the inferior olive (Text-fig. 3: 4-6).

Some bundles of degenerating pyramidal fibres passed caudalward in the medial lemniscus. The number of these fibres, although variable from case to case, was in general rather small.

In the pons, in accordance with the findings in transverse sections, degenerating fibres passed through the pontine nuclei into the tegmentum (Text-fig. 3: 4-6).

(c) Experiments of categories 5-10, with brain stem cut in transverse sections

In order to investigate the possibility that the fibres to the trigeminal region and the region ventral to and in the hilus of the nuclei cuneatus and gracilis have at



Text-fig. 5. Diagrammatic illustration of various cortico-bulbar connexions as described in the text. Abbreviations, see p. 218.

least in part their origin in the so-called motor area of the cortex, small superficial parts of respectively the face, forelimb and hindlimb areas of this region (Text-fig. 1) were removed under guidance of the map of the cat's motor cortex worked out by Garol (1942). In these experiments the same general pattern of degeneration was observed as in the experiments of categories 1, 2, 3, and 4.

Also, information could be obtained regarding differences in intensity of the fibre connexions from different parts of the motor cortex, although less clear-cut as a result of the differences in surface extent of the lesions as well as of unavoidable individual differences in impregnation intensity.

In experiment C₉ with a small lesion in the face area (Text-fig. 1) more degenerating elements were observed in the trigeminal region and especially its rostral half than in any of the other experiments. On the other hand, in experiments C₁₂ and C₁₃ with a lesion in the hindlimb area (Text-fig. 1) very few degenerating fibres and end-ramifications were present in this region, less than in any of the other experiments with lesions in the motor cortex. In addition, the large lesion of the forelimb area of experiment C₅ (Text-fig. 1), although larger than the lesion in the face area of experiment C₉, produced less degeneration in the trigeminal region especially as far as the rostral half is concerned. The above observations indicate that, although all major subdivisions of the motor cortex distribute fibres to the trigeminal region, the largest number of these corticofugal fibres, especially to the rostral half of this region, originate in the face area of the motor cortex. Moreover, in experiment C₈, with a lesion largely restricted to the premotor area (Text-fig. 1), hardly any degeneration could be observed in the trigeminal region, in contrast with experiment C₇ (Text-fig. 1) with a similar lesion which encroached upon the motor cortex and in which a moderately large number of degenerating elements were present in this region. These observations suggested that the rostral limit of the cortical area projecting to the trigeminal region corresponds roughly to that of the motor cortex. The cortical distribution of this connexion is indicated by stippling in Text-fig. 4a.

The differences in intensity of the fibre connexions from different parts of the motor cortex to the region just ventral to and in the hilus of the nuclei cuneatus and gracilis were much more difficult to evaluate.

Regarding the fibre connexions to the region at the ventral aspect of the nucleus cuneatus the following observations were made. In all the experiments with lesions in the motor cortex proper, degeneration was present in this area. Minor differences in the amount of degenerating fibres were observed in different experiments, but were difficult to evaluate due to the difference in size of the lesions. For example, a lesion in the face area in experiment C₉ produced slightly more degeneration than the lesions in the hindlimb area C₁₂ and C₁₃. This could indicate that the face area distributes more fibres to this region than the hindlimb area. In addition, the amount of degeneration produced by lesions in the hindlimb or face area was less than produced by a large lesion in the forelimb area C₅. These observations suggest that all the major subdivisions of the motor cortex distribute a good number of fibres to the region at the ventral aspect and in the hilus of the nucleus cuneatus, but especially the forelimb and perhaps also the face area, as indicated by stippling in Text-fig. 4b.

Regarding the fibre connexions to the region at the ventral aspect and in the depth of the hilus of the nucleus gracilis, degeneration could be observed in all the experiments with a lesion in the motor cortex proper. However, a maximal amount of degeneration was present in the experiments C₁₂ and C₁₃ with a lesion in the hindlimb area. Even a large lesion in the forelimb area C₅ produced obviously less degeneration in this region whilst in experiment C₉ with a lesion in the face area only very few degenerating fibres were observed. These observations suggest that the corticofugal fibre connexions to the region at the ventral aspect and in the depth of the hilus of the nucleus gracilis have their origin in all the subdivisions of the motor cortex, with a maximal intensity in the hindlimb area, decreasing gradually in the forelimb and the face area respectively. The cortical distribution of the origin of these connexions is indicated by stippling in Text-fig. 4c.*

These experiments also furnished some information regarding the other corticofugal connexions. Concerning the fibre connexions to the central tegmental area of especially the intermediate region of the medulla oblongata, it was observed that in all the experiments degeneration was present in this area. Even in experiments C₈ (Text-fig. 1) in which the motor cortex itself was hardly damaged, degenerating fibres and end-ramifications could be observed in this area, although hardly any degeneration was present in the spinal trigeminal nucleus and its vicinity. Moreover, the amount of degeneration produced, e.g. in experiment C₁₀ (Text-fig. 1) in this area was about half of that produced in experiment C₈, although both lesions were approximately of the same size. Such observations suggest that all parts of the motor cortex distributed fibres to the central tegmental area of especially the intermediate region of the medulla oblongata, but that the part of the pre-motor area covered by experiment C₈ represents an important additional source of this connexion, as indicated diagrammatically in Text-fig. 4d.

Concerning the connexions with the processus tegmentalis pontis of Bechterew, it can only be stated that these are probably established by all parts of the cortex covered in these experiments, without outstanding dominance of either of these cortical areas. However, in regard to the connexions with the magnocellular division of the lateral reticular nucleus, the impression was gained that certain subdivisions of the motor cortex projects to different parts of the lateral reticular nucleus. This will be the subject of further study.

(d) Aberrant pyramidal bundles

Throughout the brain stems in these experiments a number of discrete bundles of degenerating fibres were sometimes noticed. These bundles were not studied in adequate detail and consequently will be mentioned briefly.

In the first place a bundle of longitudinally coursing degenerating fibres was observed rather frequently in the medial lemniscus usually on the ipsilateral side, but occasionally contralateral to the lesion. Once a heavy bundle was observed which left the pyramidal tract at the transition of pons and medulla oblongata,

* It is probable that the rostral limit of the cortical area projecting to the region at the ventral aspect of the nuclei cuneatus and gracilis corresponds roughly to that of the motor cortex, as observed in regard to the connexions to the trigeminal region. However, the available experimental data are not yet as convincing.

crossed the midline and passed caudalwards in the contralateral medial lemniscus. These fibres in the medial lemniscus generally rejoined the pyramidal tract at more caudal levels.

Another category of aberrant bundles left the degenerating pyramidal tract at the level of the intermediate region of the medulla oblongata, to pass over the top of the intact pyramidal tract into the most basal parts of the opposite half of the brain stem. After crossing the midline they smoothly bent caudalwards and coursed ventral to the trigeminal nucleus all the way down into the lateral funiculus of the cervical spinal cord. These bundles are presumably a component of the pyramidal tract, which crosses at more rostral levels than the bulk of the tract and were especially clear-cut in two experiments involving hemispherectomy.

Another group of such bundles was observed at the surface of the caudal part of the medulla oblongata. These bundles left the degenerating pyramidal tract to course as external arcuate fibres over the surface of the ipsilateral as well as the contralateral side of the medulla oblongata, and at more rostral levels reached the dorsolateral and dorsal aspect of the brain stem, whence they were not traced further. Some of these bundles were observed to bend caudalwards over the lateral surface of the spinal cord.

Finally, aberrant bundles were also noticed in the region ventral to the nuclei cuneatus and gracilis and medial to the caudal part of the spinal trigeminal nucleus. These bundles were seen to leave the pyramidal decussation in the rostral direction, coursing ventral to the nuclei cuneatus and gracilis and either entering these nuclei to reach the dorsal surface of the brain stem or bending dorsalwards around their rostral pole.

It should be pointed out that the aberrant bundles are very circumscribed and contained relatively thick fibres, quite contrary to the cortico-bulbar connexions described previously, which are far more diffuse both in arrangement and distribution.

In summary (Text-fig. 5), after destruction of one hemisphere as well as after extensive lesion of the frontal pole, the following pattern of degeneration was observed in the pons and medulla oblongata:

1. A diffuse distribution of degenerating fibres from the pyramidal tract into both halves of the medulla oblongata, with a prevalence of contralateral distribution within the caudal region and a progressively more bilateral spread in more rostral regions. The most massive exit of fibres from the pyramidal tract, supplying especially the central tegmental area, was observed in the intermediate region of the medulla.

2. An accumulation of degenerating elements in the pontine grey and the processus tegmentalis pontis of Bechterew ipsilateral to the lesion and a diffuse bilateral distribution of degenerating fibres to the tegmentum of the pons, more specifically on the ipsilateral side.

3. An accumulation of degenerating elements in the spinal trigeminal nucleus and the adjoining parts of the tegmentum, in the rostral part bilateral but almost exclusively contralateral at more caudal levels, and furthermore in the region just ventral to and in the hilus of the contralateral nuclei cuneatus and gracilis. This accumulation of degenerating elements which was continuous with the degeneration

in the basal parts of the posterior horn of the spinal cord, is derived from trans-tegmental as well as recurrent pyramidal fibres. A descending limb of the recurrent system extends caudalward into the basal parts of the posterior horn, paralleling the lateral cortico-spinal tract.

4. An outstanding accumulation of degenerating elements in the magnocellular division of the lateral reticular nucleus, predominantly contralateral to the lesion.

5. Finally, in the pons, the medulla oblongata as well as the upper cervical spinal cord, very few, if any, degenerating elements were present in the motor nuclei of the cranial nerves or in the ventral horns.

6. Four groups of aberrant pyramidal bundles were observed.

The same general pattern of degeneration was produced by small lesions in the motor cortex. The degeneration in the rostral half of the trigeminal region was most pronounced following destruction of the face area of the motor cortex. Regarding the degeneration in the region at the ventral aspect of the nucleus cuneatus, this was most abundant in lesions of the forelimb and perhaps also in the face area, whereas lesions in the hindlimb area produced less degeneration. However, lesions in the hindlimb area produced a maximal degeneration at the ventral aspect and in the depth of the hilus of the nucleus gracilis. On the other hand, a lesion in the pre-motor region of the anterior sigmoid gyrus and gyrus proreus (Text-fig. 1: C₈) produced hardly any degeneration in the trigeminal region, but was followed by an especially pronounced degeneration in the central tegmental area of the intermediate region of the medulla oblongata. The amount of degeneration in other tegmental areas did not exhibit outstanding differences in intensity which could be related to differences in the localization of the lesion, except for the lateral reticular nucleus which may receive cortical projections of a somatotopic arrangement.

IV. DISCUSSION

A discussion of the literature pertinent to the present observations will be limited largely to the studies done on the cat. The literature concerned with cortical projections to the pons and the medulla oblongata is limited. From a perusal of the available data it appears that in the cat, only Redlich (1897) has observed the Marchi degeneration of a few corticofugal fibres leading from the pyramidal tract to the region of the nuclei of the dorsal funiculus. On the other hand, Nisino (1940) indicated (see his fig. 14) the presence of a few degenerating fibres in this region, following destruction of the lateral surface of the hemisphere in a monkey. Moreover, degenerating fibres to the spinal trigeminal nucleus in the monkey, following destruction of the motor area, were reported by Mettler (1935). Recently Rossi and Brodal (1956), in a footnote, mentioned the appearance of degenerating preterminals in the spinal trigeminal nucleus and the adjacent reticular formation in the cat after cortical lesion but were unable to analyse this connexion in more detail. As concerns the apparently quite extensive cortical projections to the lateral reticular nucleus, no pertinent information could be obtained from the literature, with the exception of Rossi and Brodal's (1956) recent mention of such a connexion.

The cortical projections to the tegmentum of the pons and lower brain stem has been studied more intensively. Cajal (1952) observed in the normal mouse an

important influx of pyramidal tract fibres into the central tegmental area of both sides at the level of the facial nucleus. Combs (1949) reported the appearance of degenerating fibres in the tegmentum of the medulla oblongata and the pons in rats, following unilateral destruction of the cerebral hemisphere. Probst (1899) observed Marchi degeneration in the cat leaving the pyramidal tract in the pons and medulla oblongata after lesions of the internal capsule. His illustrations correspond quite accurately with our observations. Recently Rossi and Brodal (1956), in approximate agreement with the present observations, described projections arising mainly in the rostral part of the cortex and distributing to the tegmentum of the pons and the medulla oblongata in the cat. Due to the large extent of the lesions, however, they could not detect the finer differentiations in origin reported in the present paper, and in addition were unable to establish the 'pyramidal' nature of these fibres.

In the monkey, corticofugal fibres to the tegmentum of the medulla oblongata were observed most recently by Krieg (1954). Of special significance among the corticofugal connexions are those to the cerebello-petal paramedian nuclei mentioned by Brodal (in Jansen and Brodal, 1954) and confirmed by the present study. In addition, cortical projections were noticed to the adjacent parts of the perihypoglossal nuclei, i.e. the nuclei of Roller, which according to Brodal and Torvik (1954) send fibres to the cerebellum and supply the same cerebellar region as do the paramedian nuclei.

Concerning the cortical projections to the inferior olive as described by Mettler (1935) in the monkey and more recently by Walberg (1956) in the cat, in the present material only a very few degenerating end-ramifications could be observed, perhaps as a result of the large number of degenerating fibres of passage. However, Snider & Barnard (1949) found electrophysiological evidence of such connexions. On the other hand, Cajal (1952) pointed out that in Golgi material of normal mice, it became evident that the pyramidal fibres which seem to pass into the inferior olive actually course through the nucleus to reach the tegmentum. This statement agrees with our observations in the cat, although a few of these pyramidal fibres may synapse in the inferior olive.

Regarding the absence of a well-developed direct corticofugal connexion with the motor nuclei of the brain stem, Cajal (1952) stated that such connexions could never be observed in his material of the brain stem of lower animals, which agrees with our observations. On the other hand, it is obviously in conflict with several authors in the older literature, amongst others Probst (1899) and Troschin (1902), who observed degenerating fibres passing to motor nuclei after cortical or subcortical lesions in the cat. Redlich (1897), however, already opposed these statements, pointing out that in the cat such degenerating fibres did not pass to the motor nuclei, but in the caudal region of the medulla could be traced to the area of the nuclei of the dorsal funiculus.

As was observed in the present material, the degenerating fibres, although coursing in the direction of the motor nuclei, actually passed to the trigeminal area and to the region of the nuclei cuneatus and gracilis. It is obvious that in Marchi material this was interpreted erroneously as evidence of direct connexions with the motor nuclei.

The aberrant pyramidal bundles, briefly mentioned in this study, are described

approximately in the same way in the cat by Swank (1934), as far as the external arcuate bundles are concerned. Rasmussen (1930) mentioned the existence of a recurrent pyramidal bundle in the cat, coursing rostrally through the area ventral to the nuclei of the dorsal funiculus. The other recurrent bundles in the region medial to the spinal trigeminal nucleus are probably analogous to the so-called Pick's bundle in human material, where pyramidal bundles are also observed in the medial lemniscus.

V. FUNCTIONAL ASPECTS

(a) *Corticofugal fibres to the tegmentum of the pons and the medulla oblongata*

In the present experiments, degenerating fibres from the pyramidal tract could be traced to the tegmentum of the pons and the medulla oblongata. Following the observations of Niemer & Magoun (1947), one of the functions of the pontine tegmentum in the cat is the facilitation of certain motor activities of the spinal cord. Besides, Brodal & Torvik (1955) were able to demonstrate anatomically descending pathways from this region to both halves of the spinal cord in the cat. The cortico-fugal fibres to this region probably transmit a cortical influence on the pontine tegmental facilitation mechanism.

The projections of the pre-motor and motor cortex to the tegmentum, especially of the intermediate region and the rostral half of the caudal region of the medulla oblongata, probably subserve another function. Magoun & Rhines (1946) observed that in the cat the central tegmental area of this region exerts an inhibitory action on certain motor activities of the spinal cord. Thus, it is not unlikely that the cortical projections to this area of the brain stem constitute a pathway along which the cortex exerts an indirect inhibitory influence upon the spinal cord. Tower's (1936) discovery that stimulation of the pericruciate cortex in the cat results in inhibition of a pre-existing extensor rigidity seems to support this assumption. In all likelihood, these cortical projections are in part identical with the fibre connexions from area 4S to this part of the tegmentum in the monkey, demonstrated physiologically by McCulloch, Graf & Magoun (1946). The exact cortical origin of this inhibitory cortical projection constitutes a further point of interest. Laughton (1928), McKibben & Wheelis (1932), Lindsley, Schreiner & Magoun (1949) and others stated that ablation of the pericruciate cortex in the cat caused extensor rigidity. On the other hand, Olmsted & Logan (1925), King (1927), and Langworthy (1928), stated that ablation of the excitable motor cortex around the cat's cruciate sulcus does not cause any extensor rigidity in the limbs, but that this phenomenon does occur following removal of the 'area frontalis' which corresponds to the most medial part of the anterior sigmoid gyrus at the dorsal aspect of the hemisphere (Olmsted & Logan, 1925). Tower (1936) observed that stimulation of parts of the classical motor cortex in the cat produces an inhibition of the extensor rigidity in that limb, which is under the influence of the stimulated part of the motor cortex. Besides, Tower identified a cortical field in the most medial part of the anterior sigmoid gyrus as the origin of a strong inhibitory influence on the extensor rigidity of predominantly the ipsilateral limbs. This inhibitory action is only slightly affected by section of the pyramids. The results of these experiments in the cat suggest that the entire classical motor cortex can activate Magoun and

Rhines' inhibitory region of the medulla, but that this inhibitory effect is especially dominant in the so-called 'area frontalis'.

Comparing these results with our observations, it is striking that although projections to the region of Magoun and Rhines' inhibitory zone could be traced from the motor cortex, such connexions appeared to originate most massively from the most medial part of the anterior sigmoid gyrus and the adjacent parts of the gyrus proreus, an area that corresponds with the 'area frontalis' of Olmsted and Logan (1925) and with the anterior inhibitory area of Tower (1936).

(b) *Cortico-fugal projections to the spinal trigeminal nucleus and its immediate vicinity*

The present experiments in the cat hardly furnished any evidence of direct cortical projections to the motor nuclei in the pons and the medulla oblongata. This finding exhibits an analogy with the apparent paucity of direct pyramidal synapses on the motor neurons in the spinal cord of the cat. Szentagothai-Schimert (1941) and Lloyd (1941) pointed out that such fibres terminate among the cells in the zona intermedia and the basal parts of the dorsal horn of the spinal cord, which could be confirmed in the present study in regard to the cervical spinal cord.

Although at the present time the corresponding intercalated cell groups serving the cranial motor neurons cannot be definitely identified, several anatomical considerations point towards the spinal trigeminal nucleus or its immediate vicinity as a possible location. First and foremost, this region is immediately contiguous caudally with the slender cell column formed by the medial parts of the nucleus proprius, zona intermedia and the basal parts of the posterior horn in the spinal cord. Thus, the existence of a continuous column of internuncial cells suggests itself, the cranial part of which would subserve the transmission of cortical impulses to the cranial motor nuclei. Furthermore, much like the basal part of the posterior horn and the zona intermedia in the spinal cord, the regions under discussion are situated in immediate proximity to prominent secondary sensory cell groups. It is interesting in this connexion to recall Lloyd's (1941) statement, that the spinal cell territories which are under the immediate influence of the pyramidal tract are largely identical to those receiving the local primary sensory influx.

Finally, an important argument in favour of our supposition is the fact that the cortical fibres to the rostral part of the spinal trigeminal nucleus and its vicinity originate mainly in the face area of the motor cortex, while the spinal trigeminal nucleus and the adjacent parts of the tegmentum have efferent connexions with the motor trigeminal, the facial and hypoglossal nuclei (Cajal, 1952).*

It must be emphasized that the above concept, at the present time, can only be tentative. The possibility that other cells, e.g. such as in the tegmental reticular formation (Cajal, 1952), act as internuncial elements between the pyramidal tract and the cranial motor nuclei, cannot be precluded. The finding that many such cortico-tegmental fibres originate outside the motor cortex, however, as well as the apparent absence of a somatotopic arrangement in this system, tend to render such an organization of the cortico-nuclear pathway less likely.

The implication of the cells of the spinal trigeminal nucleus and the adjacent part of the tegmentum in the cat's cortico-nuclear pathway is supported by the

* Lesions in the trigeminal area produced a profuse terminal degeneration in those motor nuclei.

physiological observations of Bremer (1923) and Magoun, Ranson & Fisher (1933). In both studies, stimulation of the face area of the motor cortex was observed to produce rhythmic masticatory movements. Magoun *et al.* could follow the corresponding pathway through the internal capsule into the cerebral peduncle and pons, but could not identify it farther caudally or in the pontine tegmentum. As stimulation of the trigeminal motor nucleus invariably resulted only in tonic closure of the jaws, the existence of a co-ordinating centre intercalated between the motor cortex and the trigeminal motor nucleus, and responsible for the masticatory rhythm, was postulated. Bremer (1923) observed that the same rhythmic chewing movements could be obtained by applying pressure and friction to the buccal commissures; pin-pricks or pinching produced no such effect. Magoun *et al.* concluded that pyramidal and trigeminal impulses appeared to influence the same 'masticatory centre'. The present data suggests that such a locus of overlap between primary trigeminal afferents and pyramidal fibres is represented by the spinal trigeminal nucleus and its immediate vicinity.

A further inference appears possible from Bremer's failure to produce masticatory rhythmicity by pin-prick stimulation of the buccal commissures. As pointed out by Olszewski (1950), the spinal trigeminal nucleus of the rabbit can be subdivided into a caudal part, which contains a pronounced substantia gelatinosa and a rostral part where the substantia gelatinosa is lacking, whereas in man there exists a transitional zone between these two subdivisions. There is suggestive evidence (Weinberger & Grant, 1943) that trigeminal pain and temperature are relayed through the caudal part of the sensory trigeminal nucleus. If this be true, then the rostral part of the nucleus could reasonably be assumed to be involved in the transmission of more strictly 'epicritic' impulses, such as those elicited by pressure and friction. It may be significant in this respect that ablation of those cortical areas from which masticatory rhythmicity can be obtained (C_9), is followed by the appearance of a more profuse degeneration in the rostral zone of the spinal trigeminal nucleus and its vicinity than can be produced by ablation of any other cortical region.

(c) *Corticofugal projections to the region at the ventral aspect and in the hilus of the nucleus cuneatus and gracilis*

At first the inference was made that the corticofugal connexions to the region at the ventral aspect and in the hilus of the nucleus cuneatus were concerned with the activity of the neck musculature. This idea was entertained especially since a primary sensory influx from the neck to this region was rather likely due to the observations of Ranson, Davenport & Doles (1932), who traced part of the fibres of the first cervical dorsal roots to the 'deeper layers of the nucleus cuneatus', and Escolar (1948) who demonstrated such connexions for at least the rostral parts of this region. On the other hand, the observations that the fibre connexions to the region of the nucleus cuneatus originate mainly in the forelimb area of the motor cortex and those to the region of the nucleus gracilis mainly in the hindlimb area indicated the possibility of another arrangement. These observations suggest that the main subdivisions of the motor cortex may influence the relay of epicritic impulses from those parts of the body which are under their special control. On a purely anatomical basis the functional implications are difficult to evaluate. It is possible, however, that the

fibre connexions to the region of the nucleus cuneatus may thus influence sensory as well as motor mechanisms, as also would seem possible for the corticofugal connexions to the trigeminal complex and its vicinity.

VI. SUMMARY

The projections of frontal parts of the cerebral hemisphere to the pons and medulla oblongata have been analysed in the cat. This was done by studying the distribution of degenerating axons and end-ramification by the aid of the Nauta-Gygax silver technique, after hemidecortication, complete frontal decortication or selective lesions of either the leg, the arm or the face area of the motor cortex. Following such lesions degenerating recurrent as well as transtegmental fibres could be traced from the pyramidal tract to the region along the ventral aspect and in the hilus of the nuclei cuneatus and gracilis, as well as to the medial part of the spinal trigeminal nucleus and the adjoining lateral parts of the tegmentum. However, a direct cortical projection to the cranial motor nuclei appeared to be lacking in the cat. The distribution area of these cortico-bulbar projections represents an immediate rostral continuation of those spinal cell groups which have been identified as the main termini of the cortico-spinal tract.

Evidence was obtained that the cortical projection to especially the rostral part of the spinal trigeminal nucleus and its vicinity originates mainly in the face area, whereas the projections to the region at the ventral aspects and in the hilus of the nuclei cuneatus and gracilis have their main origin in the forelimb or hindlimb areas respectively.

Additional frontal projection to the opposite lateral reticular nucleus could be identified, as well as diffuse bilateral projections to the central tegmental region of the middle third of the medulla oblongata, and to the paramedian nuclei and Roller's nucleus. Degenerating fibres could also be followed to the pontine tegmentum. The projections to the central tegmental region were found to arise mainly in the 'premotor' area of the anterior sigmoid gyrus and gyrus proreus.

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ADDENDUM

Since this paper was submitted for publication, Brodal, Szabo & Torvik (1956) (*J. comp. Neurol.* **106**: additional volume, 527-555), have described cortical projections to the trigeminal main sensory nucleus and to the spinal trigeminal nucleus, including the substantia gelatinosa. Additional cortical projections were described by these authors to the nucleus of the solitary tract. Despite a thorough re-examination of our material, no degeneration could be detected in either the main sensory nucleus or the substantia gelatinosa of the trigeminus. Furthermore,

a comparison of Brodal *et al.*'s illustrations with our own charts suggests that the degeneration which they locate in the nucleus of the solitary tract might correspond in part to the degeneration immediately ventral and in the hilus of the nuclei cuneatus and gracilis in the present study. Similar opinions are expressed by Chambers & Hiu, *J. comp. Neurol.* in the press.

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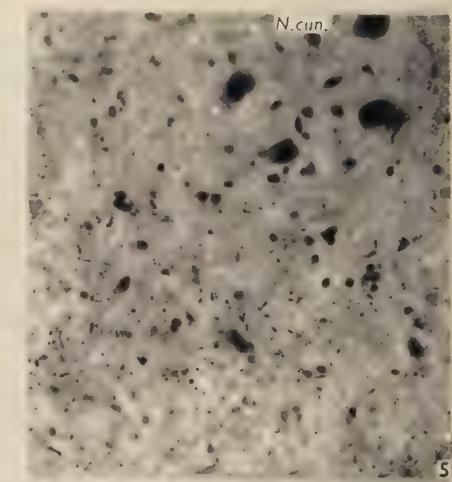
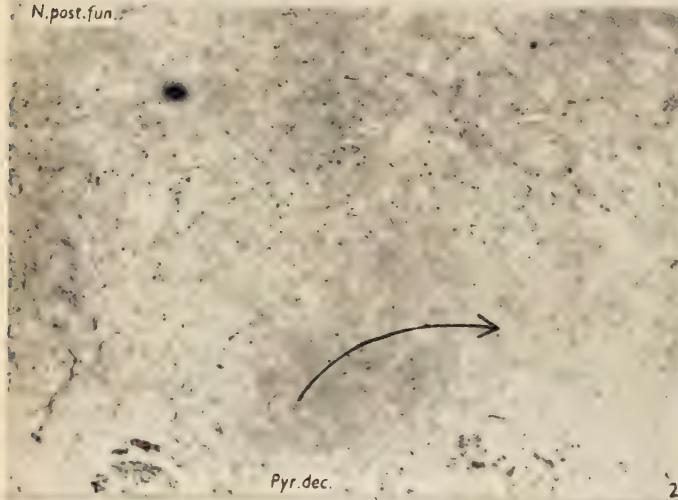
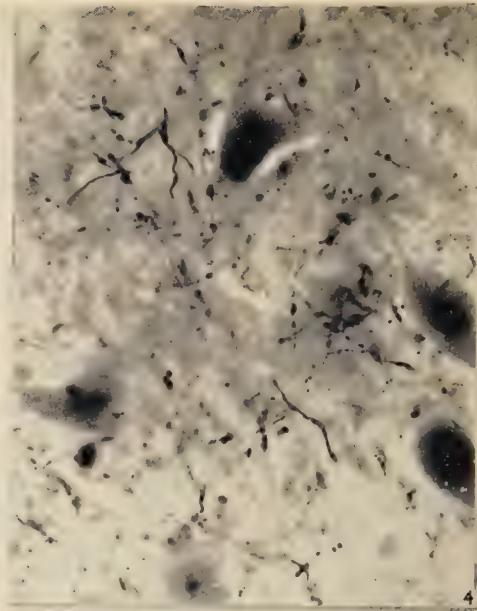
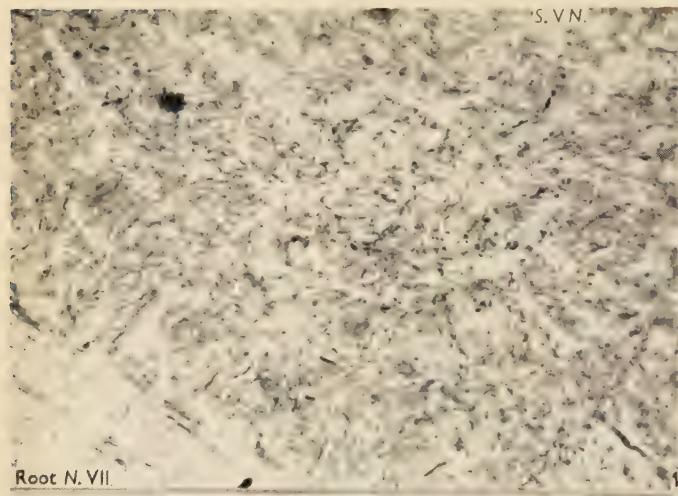
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EXPLANATION OF PLATE

Fig. 1. Degenerating fibres in the trigeminal region in experiment C₄. Impregnated according to Nauta & Gygax (1954). $\times 42.8$ (approx.).

Fig. 2. Degenerating recurrent pyramidal fibres in a sagittal section, passing from the pyramidal decussation (lower part of the field) into the region along the ventral aspects of the nuclei cuneatus and gracilis (upper part of the field). Impregnated according to Nauta & Ryan (1952). $\times 42.8$ (approx.).

Fig. 3. Degenerating fibres and end-ramifications in the trigeminal region in experiment C₄. Impregnated according to Nauta & Gygax (1954). $\times 342.8$ (approx.).

Fig. 4. Degenerating fibres and end-ramifications in the lateral reticular nucleus in experiment C₄. Impregnated according to Nauta & Gygax (1954). $\times 342.8$ (approx.).

Fig. 5. Degenerating fibres and end-ramifications in the region ventral to the nucleus cuneatus in experiment C₆. Impregnated according to Nauta and Gygax (1954). $\times 200$.

Fig. 6. Degenerating fibres on their way to the spinal trigeminal nucleus, passing over the top of the superior olive. Impregnated according to Nauta & Ryan (1952). $\times 342.8$ (approx.).

ABBREVIATIONS

<i>A.F.</i>	Anterior funiculus	<i>N. VII.</i>	Facial nucleus
<i>A.H.</i>	Anterior horn	<i>VII.</i>	Facial nerve
<i>A.N.</i>	Ambiguous nucleus	<i>VIII.</i>	Octavus nerve
<i>B.C.</i>	Brachium conjunctivum	<i>N. IX</i>	Motor nucleus of the glosso- pharyngeal nerve
<i>B.P.</i>	Brachium pontis	<i>N. X.</i>	Dorsal motor nucleus of the vagus nerve
<i>D.L.R.P.F.</i>	Descending limb of recurrent pyra- midal fibres	X	Vagus nerve
<i>I.O.</i>	Inferior olfactory nucleus	<i>N. XII</i>	Hypoglossal nucleus
<i>L.C-S.T.</i>	Lateral cortico-spinal tract	XII	Hypoglossal nerve
<i>L.R.N.</i>	Lateral reticular nucleus	<i>P.D.</i>	Pyramidal decussation
<i>M.L.</i>	Medial lemniscus	<i>P.F.</i>	Posterior funiculus
<i>M.N.V.</i>	Motor nucleus of the trigeminal nerve	<i>P.N.</i>	Pontine nuclei
<i>M.S.V.</i>	Main sensory nucleus of the trigeminal nerve	<i>P.R.N.</i>	Paramedian reticular nuclei
<i>N.B.</i>	Processus tegmentalidis pontis of Bechterew	<i>P.T.</i>	Pyramidal tract
<i>N.C.</i>	Nucleus cuneatus	<i>R.B.</i>	Restiform body
<i>N.C.E.</i>	Nucleus cuneatus externus	<i>R.P.F.</i>	Recurrent pyramidal fibres
<i>N.G.</i>	Nucleus gracilis	<i>S.G.</i>	Substantia gelatinosa
<i>N.L.L.</i>	Nucleus of the lateral lemniscus	<i>S.N.</i>	Nucleus of the solitary tract
<i>N.P.</i>	Nucleus praepositus	<i>S.O.</i>	Superior olfactory nucleus
<i>N.P.F.</i>	Nuclei of the posterior funiculus	<i>S.T.</i>	Solitary tract
<i>N.R.</i>	Nucleus of roller	<i>S.N. V.</i>	Spinal trigeminal nucleus
<i>N. VI.</i>	Abducens nucleus	<i>V.</i>	Trigeminal nerve
<i>VI.</i>	Abducens nerve	<i>T.B.</i>	Trapezoid body
		<i>V.C.</i>	Vestibular complex

GLEES STAINING OF THE MONKEY HYPOTHALAMUS : A CRITICAL APPRAISAL OF NORMAL AND EXPERIMENTAL MATERIAL

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In a recent paper, Cowan & Powell (1956) have drawn attention to certain appearances in the brain of monkey and man when stained with the Glees silver technique. They have found in their material, which they believe to be normal, a 'terminal degeneration' in septal and hypothalamic areas with a distribution in these regions resembling that seen after lesions in the olfactory bulb (Le Gros Clark & Meyer, 1947; Meyer & Allison, 1949; Adey, 1953), the amygdaloid nuclei (Adey & Meyer, 1952a), the frontal lobe (Meyer, 1950; Wall, Glees & Fulton, 1951) and the hippocampus (Simpson, 1952).

As we had not encountered this appearance in our own normal material, it seemed desirable to attempt an assessment of the incidence of such pseudodegeneration in normal preparations stained by the Glees method. A larger number of brains has been examined here than in the series of one human and four monkey brains studied by Cowan & Powell, particularly as their material included an animal from which an eye had been recently removed, another which had died of an enteric infection, and a human brain fixed without perfusion. Their published figures do not indicate from which of the monkey brains the sections had been prepared.

Apart from the question of artefacts, the relative merits of Glees and Nauta methods of displaying degeneration in the central nervous system have been the subject of conflicting opinions. Thus Evans & Hamlyn (1956) concluded that the finest degenerating ramifications, shown by the Glees technique, were not stained in Nauta preparations. They considered the Glees method satisfactory for studying the exact site of termination of axonal arborizations in appropriate regions, but that it was at a disadvantage in the presence of scanty degeneration amongst a mass of normal fibres. By contrast, Blackstad (1956) has maintained that in his studies of rhinencephalic systems, the Glees method often completely failed to display terminal structures, although pre-terminal degenerating fibres were well shown.

The disclosure by Cowan & Powell (1956) of a selectively distributed pseudodegeneration might well be a salutary warning of the difficulties which may arise in studies of degeneration by silver impregnation techniques, since, if it were found to occur even occasionally in the hands of earlier workers with the Glees method in rhinencephalic and hypothalamic systems, the results must be seriously questioned. We have unsuccessfully endeavoured to display pseudodegeneration as described

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by Cowan & Powell in normal monkey brains, and have therefore re-examined both the distribution of hypothalamic degeneration following amygdaloid and frontal cortical damage and also the effects of a dummy operation.

MATERIAL AND METHODS

A total of thirty-four monkey brains and one baboon brain have been used to date in this study. It is a pleasure to acknowledge the helpful co-operation of the Commonwealth Serum Laboratories in supplying the monkey material. All animals were immature (weighing 7–10 lb.) and in good health at the time of killing, and had been in the laboratory environment for a minimum period of 8 weeks and had received a diet containing antibiotics.

All animals except one (Rh 8) were perfused with a solution of 10% commercial formalin (non-neutralized) in normal saline. The perfusion was performed through an incision in the left ventricle which permitted insertion of the cannula into the ascending aorta. A drainage incision was made in the right atrium, and the circulation was first flushed with normal saline until the return through the atrial incision was pale pink. Approximately 400 ml. of formalin solution was then injected under a pressure varying from 110 to 120 cm. of fluid in the course of the perfusion. After removal, all brains have been fixed in 10% aqueous formalin solution for periods from 3 weeks to 9 months prior to histological examination.

Although sixty-seven animals were perfused under ether anaesthesia immediately following the aseptic removal of both kidneys for virological investigations, only twenty-one of these brains (ten *Macacus cynomolgus* and eleven *M. rhesus*) have been examined histologically. No attempt at selection has been made in the brains taken from this series for histological examination. A further series of seven *M. rhesus* monkeys were similarly perfused under deep Nembutal anaesthesia and their brains examined histologically.

In a further series of six monkeys (all *Macacus rhesus*) experimental ablations and control procedures were performed. In two, amygdaloid lesions were made, and in two others frontal lesions were inflicted. A dummy operation, involving removal of a large fronto-parietal bone flap without opening the dura mater was performed in a fifth animal. The brain of the sixth animal was examined without any prior surgical interference, in order to test the remote possibility that the pseudodegeneration might be correlated with tuberculous infection, since all six animals in this series had been culled from the colony as positive tuberculin reactors. These animals were all perfused under Nembutal anaesthesia. An extensive post-mortem examination performed by the staff of the Commonwealth Serum Laboratories failed to disclose tuberculous lesions in any of these animals.

One adult female baboon (*Papio ursinus*) was perfused under deep Nembutal anaesthesia and the brain examined histologically. Thus, in summary, histological investigations have been carried out in twenty-eight normal monkey brains and in one normal baboon brain. One monkey brain from a positive tuberculin reactor has been examined. Experimental procedures have been performed in five monkeys, and of these, one amygdaloid and one frontal ablation and a dummy operation have so far been submitted to histological examination.

Histological procedures. While it is hoped eventually to investigate with the material currently available such factors as varying impregnation and precipitation, which indubitably beset the Glees method, it was considered paramount to adhere essentially to the method used in previous studies of hypothalamic connexions of frontal, temporal and olfactory structures (Sprague & Meyer, 1950; Adey & Meyer, 1952a, b; Adey, 1953; Adey, Merrillees & Sunderland, 1956).

All brains have been sectioned frozen for Glees and Nauta preparations, using a dural freezing block, in which dry ice is evaporated in 70% alcohol. This method produces very even freezing and permits serial section of the whole diencephalon at 15μ thickness. Facilities were available for the arrangement of 450 frozen sections in sequence.

All twenty-eight normal brains have been examined by the Glees and Nauta methods. The Nauta method used was that described by Nauta & Gygax (1954). For the Glees staining, separate batches of sections were stained by two of us (W.R.A. and A.F.R.). A third series of Glees sections were prepared in seven brains (N.J.H.). Nauta staining was performed by one investigator (A.F.R.). In addition, Bodian (1936, 1937) impregnations were prepared by one investigator (I.F.H.) in twenty-one of the twenty-eight normal brains, including the seven animals perfused under Nembutal anaesthesia. The protargol used here was Protargol S (New Commission Certified—Winthrop Stearns). Except for the variations mentioned below, the Glees method followed that described by Adey & Meyer (1952b). In all, 397 Nauta sections and 677 Glees sections have been prepared in normal and control material, and an additional 125 Nauta and 70 Glees sections have been stained in experimental material, a total of 1269 sections.

Modifications tested in the Glees technique

Only such minor variations as might contribute to the appearance of pseudo-degeneration have been examined here.

(i) *Addition of buffering substances to formalin/tap water mixtures.* The local water supply is of a high order of softness, with low inherent buffering capacity. Thus in the preparation of formalin/tap water mixtures, the effects of adding citric acid or ammonium hydroxide were tested. Commercial formalin neutralized with excess calcium carbonate was used in these solutions. One to six drops of 1% citric acid were added to each 10 ml. of formalin/tap water mixture. In the two brains in which citric acid was added to the formalin solution (Rh3, Cy5) background staining was perceptibly paler and the intensity of impregnation of individual fibres proportionately reduced. Due to the reduced intensity of impregnation, fibres appeared brown in many areas, rather than the usual brownish black.

Ammonium hydroxide was used much more widely in the formalin/tap water mixtures, and in almost every batch of sections one to six drops of ammonia solution (analytical, reagent) were added to each 10 ml. of the formalin/tap water mixture, sometimes in the passage of the early sections to the ammoniated silver bath and particularly in the subsequent final reduction. This variation undoubtedly helped in reducing background staining without perceptible modification of either intensity of impregnation or morphology of the fibre elements in the hypothalamus or elsewhere.

(ii) *Variations in methods of fixation.* Since variations in osmotic interrelationships and relatively slow fixation might account for the beaded appearances seen in pseudodegeneration, particular note was taken of the adequacy of fixation at the time of removal of the brain. Persistence of pinkish areas and the presence of blood in the surface vessels was regarded as evidence of defective fixation. The assessment of fixation is presented in tabular form (Table 1).

Table 1

Brain	Anaesthetic	Quality of perfusion	Quality of fixation	Silver impregnation methods and staining quality		
Rh 1	Ether	Poor	Poor	G ++	N ++	B ++
Rh 2	Ether	Good	Good	G ++	N ++	B ++
Rh 3	Ether	Poor	Medium	G + + +	N ++	B + + +
Rh 4	Ether	Good	Good	G +	N ++	B ++
Rh 5	Ether	Good	Good	G poor	N +	B poor
Rh 6	Ether	Good	Good	G ++	N ++	B ++
Rh 7	Ether	Good	Excellent	G poor	N +	B +
Rh 8	Ether	Unperfused	Fair	G ++	N ++	B ++
Rh 20	Ether	Good	Excellent	G ++	N ++	
Rh 21	Ether	Good	Good	G ++	N ++	
Rh 11	Ether	Excellent	Excellent	G + + +	N ++	
Rh 22	Nembutal	Good	Excellent	G + + +	N ++	B + + +
Rh 23	Nembutal	Good	Good	G ++	N ++	B + + +
Rh 24	Nembutal	Good	Good	G ++	N ++	B + + +
Rh 25	Nembutal	Poor	Inadequate	G + + +	N ++	B + +
Rh 26	Nembutal	Fair	Fair	G ++	N ++	B ++
Rh 27	Nembutal	Poor	Inadequate	G ++	N +	B + + +
Rh 28	Nembutal	Poor	Inadequate	G +	N +	B + +
Cy 1	Ether	Moderate	Moderate	G ++	N ++	B ++
Cy 2	Ether	Good	Good	G +	N ++	B ++
Cy 3	Ether	Good	Good	G + + +	N ++	B ++
Cy 4	Ether	Good	Excellent	G poor	N ++	B ++
Cy 5	Ether	Poor	Poor	G ++	N ++	B ++
Cy 6	Ether	Moderate	Moderate	G variable	N ++	B ++
Cy 7	Ether	Good	Excellent	G variable	N ++	B ++
Cy 11	Ether	Good	Fair	G ++	N ++	
Cy 16	Ether	Fair	Poor	G +	N ++	
Cy 17	Ether	Fair	Poor	G +	N ++	

An arbitrary assessment of general staining quality from poor to three-plus has been used in these normal brains in relation to each staining method tested, Glees (G), Nauta (N) and Bodian (B).

To estimate the effects of agonal and autolytic changes, one brain (Rh 8) was left unperfused. It was removed 2 hr. after death and placed in 10% aqueous formalin solution for 11 days. Subsequent Glees staining of the diencephalon produced adequate impregnation of hypothalamic fibres, although the degree of impregnation varied more from place to place in a given section than in the perfused material. There was some precipitation along the course of individual fibres, apparently of adherent metallic particles, but no appearances likely to be construed as pseudodegeneration could be detected.

The effects of storage of cut frozen sections have been examined in one brain (Rh 7). Separate batches of sections were kept for 3 days after cutting in 10% formalin solution and in 10% formol saline, prior to transfer to ammoniacal alcohol in the Glees procedure. Impregnation after storage in formalin solution was poor, with heavy precipitation. No beading of fibres was seen. Better impregnation followed storage in formol saline solution, but gross beading of fibres occurred

diffusely in many areas of the hypothalamus and elsewhere in the diencephalon. This beading involved large fibres without interruption of continuity and showed no pattern of distribution. The appearance of the fibres, though abnormal, and the diffuse nature of the change, can scarcely lead to confusion with the picture of true degeneration, particularly as no beading could be seen amongst the terminal plexuses in the hypothalamus or thalamus.

A striking difference was seen in the intensity of impregnation of the fine pericellular plexuses in the hypothalamus in the Protargol-stained material when comparisons were made between animals killed under ether and Nembutal. A much more intense impregnation uniformly followed perfusion under Nembutal than with ether. These differences could not be discerned in similar comparisons of Glees and Nauta preparations from the two groups of brains. However the results with the Protargol method confirm the widely held opinion that the use of volatile anaesthetics may exert a deleterious effect on subsequent silver impregnation of neural tissue.

(iii) *Variations in the technique of Glees silver impregnation.* Since the cut sections are washed briefly in water as the initial step in the Glees method, prior to being transferred to ammoniacal 50% alcohol for 48 hr., we have tested the effect of a period of 24 hr. immersion in water on the sections from one brain (Rh 27). Frozen sections were cut on the 35th day after perfusion, which was imperfect in posterior thalamic areas. Subsequent Glees staining was good in almost all sections, with little precipitation and no significant beading in any part of the diencephalon.

A similar procedure was tested in sections from one brain (Rh 26) by extending the period in water to 24 hr. after removal from ammoniacal alcohol and prior to impregnation with 10% silver nitrate. Good staining followed in these sections. In comparison with sections from the same brain not so treated in water, but stained at the same time, the larger fibres in the lateral hypothalamic and thalamic areas showed a high proportion of 'knobs', darkly stained swellings along the course of the fibres which did not appear to be associated with modified structure or staining of the intervening portions of the axon. This appearance was found diffusely through the section. No beading or disruption of finer axonal structures in pericellular plexuses could be detected.

Prolonged exposure to 10% silver nitrate solution does not appear to exert a significant effect on the appearance of the stained fibres. Sections from the brain C2, which had been subjected to a dummy operation (*vide infra*) were placed in 10% silver nitrate for 63 days and stored in the dark. By this time they had taken on a dark chocolate-brown colour. They were bleached in 1% sodium thiosulphate solution for 15–30 sec. and washed in five changes of water. They were then returned to 10% silver nitrate. Batches of sections were stained in the normal way 2½, 5½ and 26 hr. later. Particularly satisfactory impregnation occurred in the bed nucleus of the stria terminalis, the hypothalamus, septum and preoptic area without evidence of pseudodegeneration.

We have also examined in two brains (Cy 6 and Cy 7) the effects of an additional water wash between the conclusion of the first formalin reduction and immersion of the section in the ammoniacal silver bath. It appeared that this procedure was followed by unpredictable variability in impregnation and an increase in both

precipitation and background staining. No appearance suggestive of pseudodegeneration was seen in these sections.

It has been found that weaker formalin/tap water mixtures than the customary 10% concentration can be used with advantage in displaying the fine fibre elements in medial hypothalamic areas. The 'development' of the final picture occurs more slowly, but usually with increased ultimate contrast, in that extremely delicate ramifications are well defined against a pale background. Suitable mixtures may have a formalin concentration between 0·5 and 3·0%.

RESULTS

A. Survey of findings in normal material

It would not seem rewarding to enter into a detailed description of fibre appearances by the Glees method in the normal diencephalon on the basis of regional differences in the morphology of fibre terminals or the arrangement of fibre bundles and pericellular plexuses. Indeed, the most careful assessment of our material does not suggest that the method would provide a fruitful basis for such a qualitative differentiation.

We may, however, draw attention to certain variations in staining noted in this study. It appears that certain difficulties attach to the adequate and consistent impregnation of the region of the median eminence and adjoining medial areas of the caudal hypothalamus. Sections which were well stained in other regions frequently remained poorly impregnated in these ventral areas, and proper staining here appeared critically dependent on such factors as the nature and amount of the buffering substances in the formalin/tap water mixtures, as outlined above.

In such poorly impregnated sections, the fibres took on a pale and ghostly appearance, and in such cases the range of contrasting densities between glial and neural fibre elements was much reduced, since the neural fibres displayed little of the argentophilia characteristic of Glees preparations. Such a confusion has also been noted in the preparations of the glial and neural elements of the neurohypophysis of the cat and rat (Green & van Breemen, 1955). In such sections, too, tiny scattered granular bodies were sometimes discerned at the limits of resolution of the light microscope. Although occasionally arranged in linear aggregates, they could scarcely be construed as representing the terminals of neural elements by the criteria currently employed, having regard to their small size, their lack of argentophilia and their diffuse distribution. These granules showed considerable resemblance to the surface precipitation of impregnating material which commonly accompanied this appearance. They showed no similarity to the larger, intensely argentophilic fragmented terminals seen previously in the dorso-medial and ventro-medial hypothalamic nuclei following amygdaloid lesions (Adey & Meyer, 1952a).

More cogent to any discussion of pseudodegeneration in Glees preparations is the occasional appearance of scattered fragmented fibres bearing all the characters of true degenerating fibres. These have been described with a variety of silver staining techniques. In our material they appeared more numerous in Bodian preparations than in either Glees or Nauta sections. A very careful search of a Bodian preparation of the whole diencephalon might reveal six to ten such fragmented fibres occurring in a completely random fashion throughout the section. In Glees material from the

same brain, a search of several sections was usually necessary to reveal a single such fibre. Their incidence in the hypothalamus was no higher than in other regions of the diencephalon. It may be suggested that the process of paraffin embedding at relatively high temperatures may account for the higher incidence of this appearance in Bodian material.

Particular attention has been directed to the appearance of fibres along the margins of sections. Prior to the present study we had observed from time to time tortuous fibres in Glees sections of normal tissues adjacent to the free surfaces of the section. The fibres so involved might be sharply torn across and separated from an adjoining segment of fibre by a short interval. They might also exhibit beaded swellings on an otherwise normally staining axon. This complicating factor in the interpretation of degeneration in such bundles as the stria medullaris has been mentioned elsewhere (Adey *et al.* 1956). It is our impression that this appearance occurs rarely in the freezing method used in the present study, involving the slow induction of a steady state of freezing with dry ice, as compared with earlier methods depending on production of a transient and highly variable frozen state with gaseous carbon dioxide. The violent rupture of marginal fibres, suggested by the microscopic appearance in such cases, supports the hypothesis that it may result from the rapid freezing and melting which is an inevitable accompaniment of the gas-freezing method. We have not seen any appearance in the present normal Glees material of periventricular structures suggesting pseudodegeneration which might conceivably have resulted from such an insult to the tissue.

We have encountered no difficulty in securing adequate and consistent impregnation of the septum, the bed nucleus of the stria terminalis, the supraoptic and preoptic areas, and have not seen any evidence of pseudodegeneration amongst either the larger fibres or fine pericellular plexuses in Glees preparations of these regions.

B. Appearances of degeneration in experimental material

In view of an inability to mimic the appearances of significant degeneration in normal material by the Glees method, we have turned to an examination of the diencephalon in a series of operated brains. The findings following an amygdaloid lesion (*Am 1*) and a frontal ablation (*Fr 1*) will be presented here, since they contrast sharply with the negative findings in the large amount of normal material examined. Two other brains, with amygdaloid and frontal lesions respectively, were prepared contemporaneously with *Am 1* and *Fr 1*, but will be examined later as part of a continuing study.

(i) *Control brain (C 1)*. Since all animals in this group were positive tuberculin reactors, this subject was used as a control to test the remote possibility that pseudodegeneration might be connected with the tuberculous infection. This animal was caged with *Am 1* and *Fr 1* preoperatively and during their post-operative survival period. Histologically, its brain showed no significant abnormality with the Glees and Nauta methods.

(ii) *Effects of dummy operation (C 2)*. In this animal a large fronto-parietal bone flap was removed under Nembutal anaesthesia. The dura mater was not opened. Five days post-operatively perfusion was performed under Nembutal anaesthesia.

No evidence of degeneration was found in the hypothalamus, septum or preoptic area. The effects of very long exposure to silver nitrate on sections from this brain have been described above.

(iii) *Distribution of degeneration following an amygdaloid lesion.* Since previous studies using both Marchi (Fox, 1940, 1948) and Glees (Adey & Meyer, 1952a) methods have indicated extensive amygdalo-hypothalamic pathways running in part at least through the stria terminalis, we have examined the diencephalon in the brain of an animal (*Am 1*) killed 6 days after an amygdaloid lesion.

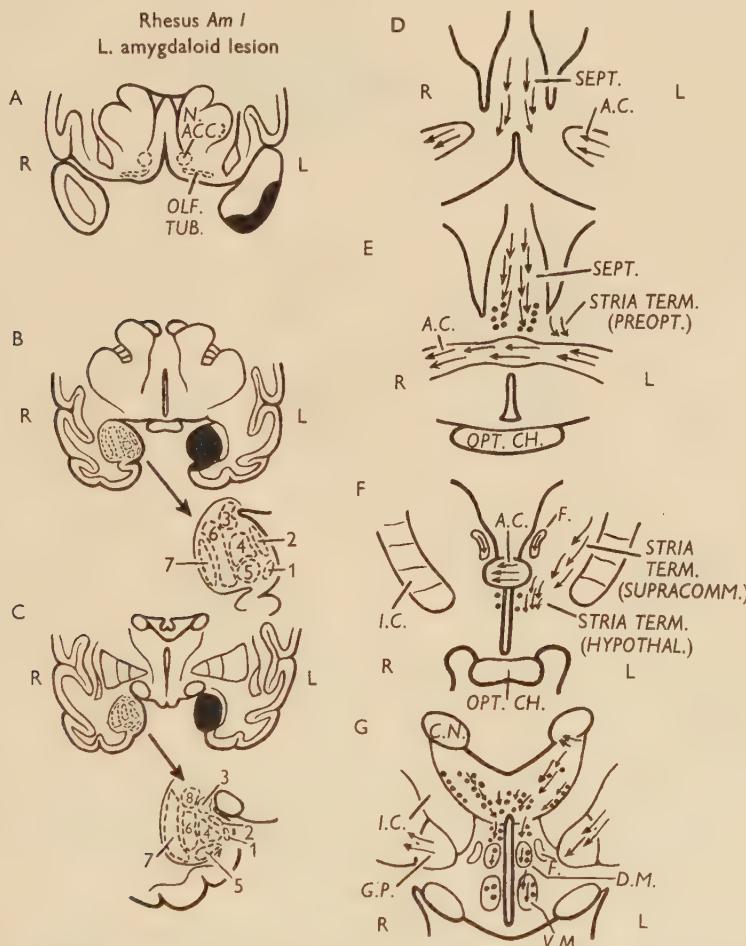
The extent of the amygdaloid involvement (Text-fig. 1 A-C) resembles that of another brain (M 85) described previously (Adey & Meyer, 1952a). Anteriorly, the lesion has destroyed the cortex of the inferior part of the tip of the temporal lobe and on the lateral aspect of the hemisphere extends for a short distance into the lower part of the superior temporal gyrus and the middle temporal gyrus. On the medial side of the temporal lobe the primary olfactory cortex in the temporal prepyriform area has been extensively resected. Further posteriorly, at the level of the optic chiasm, the lesion has removed the anterior portion of the entorhinal cortex. In the overlying amygdala, the medial and most of the lateral parts of the basal nucleus, the cortico-amygdaloid transition area and a portion of the lateral nucleus have been removed. Only the medial nucleus, parts of the lateral nucleus, and the dorsal divisions of the basal nucleus appear to have been spared at this level. Caudally the lesion is sharply circumscribed within the anterior entorhinal cortex and overlying amygdaloid nuclei, including the entire basal complex, but sparing the central nucleus, the medial nucleus and a narrow strip of the lateral nucleus. The anterior end of the hippocampus is not involved.

Not only has the examination of this brain confirmed the general distribution of hypothalamic degeneration described in M 85 by Adey & Meyer following an amygdaloid lesion, but new data has also been gleaned concerning certain additional pathways from the temporal lobe to the hypothalamus. Examination of both Glees and Nauta sections at each level has served to emphasize the merits of each method in different situations.

Nauta sections at the level of the anterior commissure showed considerable numbers of degenerating fibres traversing the commissure (Text-fig. 1 E). These fibres were widely dispersed through all dorso-ventral levels of the commissure (Pl. 1, fig. 3). They could be traced across the mid-line to the contralateral limits of the commissure, and a few appeared to leave it dorsally and enter the lateral part of the base of the septum on both sides of the mid-line. In Glees sections at the same level, the degenerating fibres tended to be obscured by the enormous numbers of heavily stained normal fibres.

Coronal sections of the septum in a plane slightly anterior to the median portion of the anterior commissure (Text-fig. 1 D) showed degenerating fibres passing downwards through the septum in a paramedian plane bilaterally (Pl. 1, figs. 1, 2). They appeared first near the upper border of the septum and extended inferiorly to the level of the ventral border of the anterior commissure. It was our impression that these fibres were more numerous on the operated side. Scattered degenerating terminals were also detected along the course of these fibres which were more strikingly displayed in Nauta than Glees preparations.

Further posteriorly, coronal sections through the middle of the anterior commissure again showed degenerating fibres coursing ventrally through the septum on both sides of the mid-line (Text-fig. 1 E). These fibres were more profuse than at the level of Text-fig. 1 D, and they appeared to arise from the fornix bundles visible in the upper part of the section. Many degenerating terminals are visible in the



Text-fig. 1. Extent of temporal lesion (A, B, C) is indicated in solid black. Secondary degeneration as seen in Nauta and Glees preparations is seen in D-G. Degenerating fibres are indicated by arrows and degeneration in terminal ramifications by dots. Abbreviations: A.C., anterior commissure; C.N., caudate nucleus; D.M., dorso-medial hypothalamic nucleus; F., fornix; G.P., globus pallidus; I.C., internal capsule; N.ACC., nucleus accumbens; OPT.CH., optic chiasm; SEPT., septum; STRIA TERM. (HYPOTHAL.), hypothalamic bundle of stria terminalis; STRIA TERM. (PREOPT.), preoptic bundle of stria terminalis; STRIA TERM. (SUPRACOMM.), supracommissural bundle of the stria terminalis; V.M., ventro-medial hypothalamic nucleus; 1, cortico-amgdaloid transition area; 2, cortical amygdaloid nucleus; 3, medial amygdaloid nucleus; 4, accessory basal amygdaloid nucleus; 5, medial part of basal amygdaloid nucleus; 6, lateral part of basal amygdaloid nucleus; 7, lateral amygdaloid nucleus; 8, central amygdaloid nucleus; OLF. TUB., olfactory tubercle.

medial septal nucleus dorsal to the anterior commissure. Further laterally at this level, scattered degenerating fibres are present in the supracommissural bundle of the stria terminalis on the operated side, but no degeneration could be detected in the commissural bundle of the stria nor could any degeneration be detected in the preoptic region ventral to the anterior commissure. The stria terminalis bundles contralateral to the lesion were normal at this level.

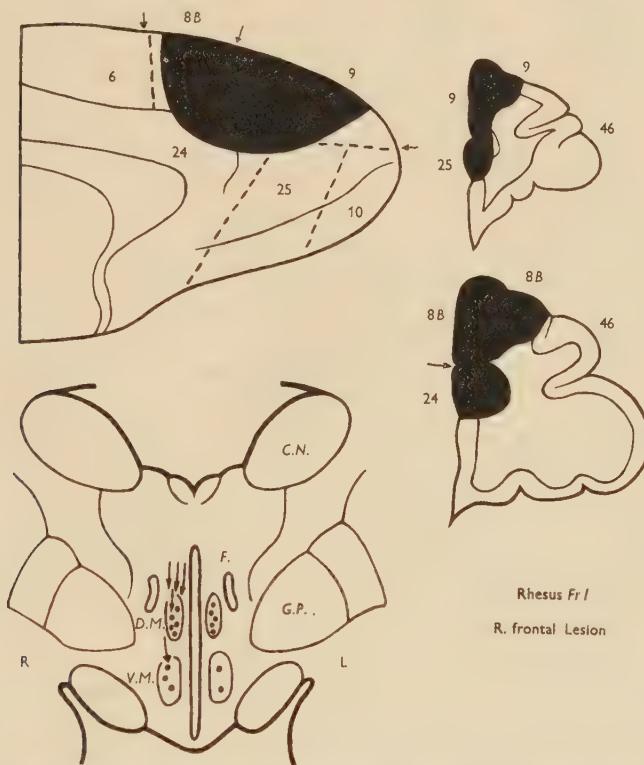
At the posterior border of the anterior commissure (Text-fig. 1 F) the hypothalamic bundle of the stria terminalis clearly displayed numerous degenerating fibres. These could be traced dorsally in the section along the medial border of the internal capsule to the ventral part of the head of the caudate nucleus, following the course of the bed nucleus of the stria terminalis (Pl. 1, figs. 7-9). At this level the fornix columns lie close to the upper border of the anterior commissure. There were scattered degenerating fibres in the fornix bilaterally (Pl. 1, figs. 4, 5). In sections slightly further forward, these fibres ran in considerable numbers at the medial border of the fornix and appeared to terminate in the medial septal nucleus. Ventral to the anterior commissure at the level of Text-fig. 1 F, the degeneration rapidly decreased, with only scattered fibres and terminals degenerating in the paraventricular nucleus. No degeneration was seen in the suprachiasmatic or supraoptic nuclei.

Further caudally (Text-fig. 1 G), examination of Nauta preparations revealed a wide distribution of degenerating fibres. On the operated side fine fascicles of degenerating fibres were found to sweep medially into the thalamus ventral to the caudate nucleus. Within the thalamus they were found mainly in the reticular nucleus adjoining the internal capsule, in the mid-line nuclei and in the intralaminar nuclei (Pl. 1, figs. 10, 11). A similar but less extensive distribution of degenerating fibres occurred in the contralateral thalamus. The involvement of the mid-line nuclei was heavy bilaterally. No degeneration was found in the caudate nuclei (apart from the fascicles at its ventral border) nor in the internal capsules. Ventrally in the hypothalamus degenerating fibres and terminals occurred bilaterally in the dorso-medial and ventro-medial hypothalamic nuclei (Pl. 1, fig. 6), but gradually decreased in amount at the more ventral zones. The lateral hypothalamus showed no degeneration on either side, nor were degenerating fibres found in the fornix on either side at this level. In the globus pallidus and lentiform nucleus on the operated side obliquely directed fascicles of fine degenerating fibres and degenerating terminals were seen. Less numerous degenerating fibres and terminals were seen in the contralateral globus pallidus (Pl. 2, figs. 12, 13).

While the Nauta preparations allowed an excellent appraisal of the general distribution of the degeneration in a large volume of tissue, it was our impression that the method might not impregnate the finest elements of a degenerating pericellular plexus. This impression was supported by our observation that degeneration in the dorso-medial and ventro-medial hypothalamic nuclei in this brain was best seen in its finest pericellular ramifications in Glees preparations (Pl. 1, fig. 6), although the search for these appearances amongst many normal fibres required considerably greater care and patience.

(iv) *Hypothalamic appearances following a frontal lobe lesion.* A right frontal cortical resection was performed under Nembutal anaesthesia in an immature macaque (*Fr 1*). The animal was perfused 6 days later under Nembutal anaesthesia.

The lesion (Text-fig. 2) mainly involved cortex on the medial side of the hemisphere in areas 8B and 9, extending inferiorly slightly into the anterior end of area 24. The lesion did not involve the frontal pole anteriorly, nor did it appear to extend significantly into area 6 posteriorly.



Text-fig. 2. Extent of frontal lesion indicated in solid black. Distribution of secondary degeneration in the hypothalamus is indicated by arrows (fibres) and dots (terminals). Abbreviations: C.N., caudate nucleus; D.M., dorso-medial hypothalamic nucleus; F., fornix; G.P., globus pallidus; V.M., ventro-medial hypothalamic nucleus.

We have confined our histological examination of this brain to Glees and Nauta sections at the level of the dorso-medial hypothalamic nuclei. Glees preparations showed degenerating fibres entering the dorso-medial nucleus from its dorsal aspect on the operated side (Pl. 2, fig. 14). There were numerous fragmented and swollen terminals in more ventral parts of the ipsilateral dorso-medial nucleus (Pl. 2, fig. 15). Similar fragmented terminals were present in the contralateral dorso-medial nucleus with a similar density of distribution (Pl. 2, fig. 16), but no degenerating fibres could be seen entering the dorsal aspect of this nucleus. In the ventro-medial nuclei many more fragmented terminals were seen on the operated than the unoperated side. Careful examination of Nauta preparations at the same level has disclosed very little fibre or terminal degeneration on either side of the hypothalamus.

DISCUSSION

While it can be unequivocally stated that this study of the normal monkey diencephalon has failed to disclose a pseudodegeneration of the type described by Cowan & Powell (1956) in preparations by either the Glees or the Nauta methods, it is disappointing that the variations in the Glees technique tried here have added little to our knowledge of how this appearance might arise. It may be conceded that such unusual procedures as absence or inadequacy of perfusion, lengthy maceration of the tissues in water at various stages of the staining, or marginal damage during the freezing process may alter the intensity of impregnation and even occasionally the fibre morphology in an unpredictable fashion, but it has not been our experience that these changes mimic in any way the appearances of true degeneration. Nor do the changes in impregnation in Bodian sections seen here after killing under ether anaesthesia produce a picture of pseudodegeneration.

Nevertheless, there is a considerable body of work published prior to the observations of Cowan & Powell (1956) supporting the presence of beaded and fragmented fibres in the infundibular region as an apparently normal phenomenon. Hagen (1952) drew attention to the factors of age and disease in initiating morphological changes in the fibre and cellular elements of the diencephalon. In an extensive investigation of the human diencephalon and hypophysis obtained from accident victims and fixed not more than 12 hr. after death, Hagen noted in Bielschowsky-Gros and Bodian preparations neurofibrillar swellings along the course of the finest axis cylinders in the hypophyseal stalk and neurohypophysis, and further, that these knotted thickenings appeared to lose continuity with nerve fibre elements, their linear arrangement then indicating the original course of the nerve fibre. Hagen drew attention to the possible confusion of these elements with degenerating nerve fibres, and considered that they represented a normal physiological process.

Subsequently Knocke (1953) examined Bielschowsky-Gros preparations of the hypophysis and diencephalon, mainly in young dogs whose brains were perfused immediately after death with 10 or 20% formalin solution. Knocke found nodular fibres in the tuber cinereum, the supraoptic and paraventricular nuclei, and in the neurohypophysis. At times the nodular fibres appeared as discontinuous fine granules, and in the peripheral parts of the infundibulum these deeply staining fibres were condensed into tracts arborising in the infundibular region and around the blood vessels of the neurohypophysis.

While fixation of the brain was not initiated in either of the foregoing investigations until after death, our results in one unperfused brain do not suggest that this is necessarily a cause of pseudodegeneration. However, neither of those investigations disclose the widespread distribution of pseudodegeneration reported by Cowan & Powell, involving the lateral septal nucleus, nucleus accumbens, bed nucleus of the stria terminalis, anterior hypothalamic areas and the region of the tuberal nuclei. A more localised distribution of this appearance in the vicinity of the tuber cinereum and adjacent supraoptic and paraventricular nuclei is suggested by both Hagen and Knocke.

It is a matter of some interest that Cowan & Powell should include as a control exhibiting typical pseudodegeneration the brain of an animal with an eye recently

removed, since previous work from the same laboratory has shown that in the ferret, at least, the normal gonadal response to visual stimulation may involve optic pathways to the subthalamus or the ventral nucleus of the lateral geniculate body (Le Gros Clark, McKeown & Zuckerman, 1938). Gonadal activation invariably followed retinal stimulation even after essentially complete bilateral section of the optic tracts at the ventral border of the dorsal nucleus of the lateral geniculate body. Other work from the same laboratory has shown that in the ferret fibres from the optic chiasm can be traced into the hypothalamus in close relation to the wall of the third ventricle (Jefferson, 1940), although it is claimed that these are aberrant fibres which eventually rejoin the optic tract. However, the Marchi technique used in that study would not disclose the presence of unmyelinated collaterals terminating in the hypothalamus.

A neurosecretory function has been ascribed to these beaded hypothalamic fibres (Hagen, 1952; Mazzi, 1954; Cowan & Powell, 1956). However, the wide distribution of the beading seen by Cowan & Powell in Glees preparations extends appreciably beyond the regions to which neuroendocrine functions are normally ascribed, and includes the septum and the bed nucleus of the stria terminalis. A more cautious attitude is taken by Green & van Breemen (1955) who noted in Gomori preparations of the rat a gradual appearance of Gomori substance in the cells of the neural lobe in the first two weeks after birth. However, the hypophyseal stalk and median eminence remained clear of Gomori material in all these preparations, a surprising finding if it is assumed that the Gomori material is being produced in hypothalamic nuclei and is passing through nerve fibres of the neural stalk to be stored in the neural lobe. Moreover, Green & van Breemen were unable to see an accumulation of Gomori material in the tractus hypophysius during recovery from dehydration or from saline administration, as might be expected on the basis of a neural secretion hypothesis.

Furthermore, Green & van Breemen concluded that, if the granule masses seen in the neural lobe by electron microscopy correspond to the Gomori substance, and if the nerve fibres resemble those seen in silver impregnations, then the granule masses are so large that they cannot possibly lie within nerve fibres. Yet the granule masses are enclosed in membranes suggesting that they lie in distorted fibres or separated parts of the parent cell. These membranes may arise from nerve fibres, pituicytes or modified ependymal cells, but at this stage their glial or neural origin cannot be settled. It is interesting that Green & van Breemen found granules in the median eminence in an intra-axonal position but these granules were only one-third the diameter of those in the neural lobe and in the Herring bodies.

Turning briefly to our findings in experimental material, which contrast so sharply with our negative results in the substantial volume of normal and control material, the brain *Am 1* with an amygdaloid lesion provides interesting evidence of direct septal connexions. Such a pathway had been suggested from electrophysiological studies (Gloor, 1955), which had revealed that the shortest latency responses to amygdaloid stimulation in the cat are recorded in the base of the septum, the nucleus accumbens, the preoptic area and the anterior hypothalamus. It is of particular interest that Gloor's studies disclose projections to the bed nucleus of the stria terminalis and to the ventro-medial hypothalamic nucleus from the amygdala, thus

supporting both the present histologic results and those described elsewhere (Adey & Meyer, 1952a).

Fox (1943) considered that fibres of the precommissural fornix of the cat terminating in the lateral septal nucleus arise in the anterior end of the hippocampus adjacent to the amygdala. Since the lesion in *Am 1* clearly spares the anterior end of the hippocampus, it would appear that these septal projections through the fornix may in fact arise in the amygdala in the monkey.

The lesion in our brain had also involved the tip of the temporal pole and adjoining cortex on the lateral surface of the temporal lobe. Electrophysiological evidence has indicated additional reciprocal projections between the temporal pole and the septal region (Stoll, Ajmone-Marsan & Jasper, 1951). Stimulation of the septal nuclei produced bilateral responses in the temporal poles, with a latency only slightly longer in the contralateral than the ipsilateral temporal tip. Similar bilateral responses in the temporal poles followed stimulation of the nucleus lateralis posterior of the thalamus. Stoll *et al.* present evidence that the connexions with the septum run in part through the fornix system. These findings are supported by the bilateral degeneration we have found so clearly displayed in Nauta sections, and affords some explanation for the difficulty experienced by Cowan & Powell (1956) in accepting such bilaterality as evidence of valid degeneration in the septum and hypothalamus. McLardy (1955a, b) has described bilateral degenerative changes in the lateral septal nucleus of the macaque following both fornix and temporal pole lesions and has produced evidence of a partial interfornix decussation in at least the septopetal component of this two-way temporoseptal system.

It is obvious that this study has not extended into many important facets of the problem of silver impregnation of neural tissue. It has been our prime purpose to test the extent to which pseudodegeneration might be seen in the indubitably normal primate diencephalon when stained by us with the Glees method, having regard to the previous use of the method by one of us in studies of the hypothalamic connexions of the temporal lobe (Adey & Meyer, 1952a). The more common difficulties which beset the use of silver impregnation techniques in the display of degenerating neural elements have been extensively discussed by Glees & Nauta (1955) and need no elaboration here. Our failure to find pseudodegeneration with the Glees method, even in such a relatively large volume of normal material, and despite a rigorous search of those hypothalamic areas to which unusual staining reactions and morphological appearances have been attributed, would seem in itself a relatively fruitless and negative conclusion to such a study. We may perhaps take heart that in experimental material our results following an anterior temporal lesion confirm in both Glees and Nauta preparations the general arrangement of hypothalamic degeneration previously reported (Adey & Meyer, 1952a). Moreover, the direct comparison possible here of Glees and Nauta preparations from the same region has led to the conclusion that each may have its place in the scheme of investigation of neural degeneration. As already suggested by Evans & Hamlyn (1956), the Glees method would seem to achieve a more extensive impregnation of the finest terminal ramifications than can be secured with the Nauta technique. On the other hand, the clarity of Nauta preparations allows immediate and easy assessment of limited degeneration in such bundles as the anterior commissure and fornix, where

heavily stained normal fibres in Glees sections readily obscure degenerating elements.

SUMMARY

1. The appearance of the diencephalon has been examined with a variety of silver staining methods in thirty-four monkey brains and one baboon brain. All animals were in good health at the time of killing. Glees, Nauta and Bodian techniques have been used in this study. A total of 1269 sections stained by the Glees and Nauta methods have been examined.

2. In unoperated and control material, comprising 397 Nauta sections and 677 Glees sections, we have been unable to discern pseudodegeneration of the type described by Cowan & Powell (1956). A particular search for this appearance has been made in the hypothalamus, bed nucleus of the stria terminalis and septal nuclei. The control material included an animal subjected to a dummy operation involving removal of a large fronto-parietal bone flap but leaving the dura mater intact.

3. Various modifications of the Glees staining procedure have been tried in unsuccessful endeavours to produce an appearance of pseudodegeneration. Ether anaesthesia prior to perfusion impaired impregnation in Bodian preparations of the hypothalamus in comparison with similar preparations from brains perfused under Nembutal anaesthesia.

4. In a brain with a unilateral amygdaloid and temporopolar lesion, we have confirmed the findings of Adey & Meyer (1952a) of bilateral terminal degeneration in the dorso-medial and ventro-medial hypothalamic nuclei, in both Glees and Nauta preparations. We have confirmed the role of the stria terminalis as a pathway to the hypothalamus on the side of the lesion. Nauta preparations in this brain disclose bilateral degeneration in septal nuclei, with bilateral degeneration in fibres of the fornix in its course adjoining the septum. Extensive bilateral terminal degeneration was seen in the dorso-medial hypothalamic nuclei following a unilateral frontal lesion. It would appear that Nauta preparations permit ready assessment of scattered degenerating fibres in such dense bundles as the fornix and anterior commissure, whereas Glees preparations display more suitably degenerating terminal ramifications in such regions as the dorso-medial and ventro-medial hypothalamic nuclei.

It is difficult to acknowledge adequately the opportunity afforded one of us (W.R.A.) under a Royal Society and Nuffield Foundation Commonwealth Bursary to discuss matters relevant to this study with other workers in this field in Great Britain.

It is a pleasure to acknowledge the assistance of Dr P. L. Bazeley, Director of the Commonwealth Serum Laboratories and members of his staff in the provision of the monkey material, and without whose assistance this study would not have been possible.

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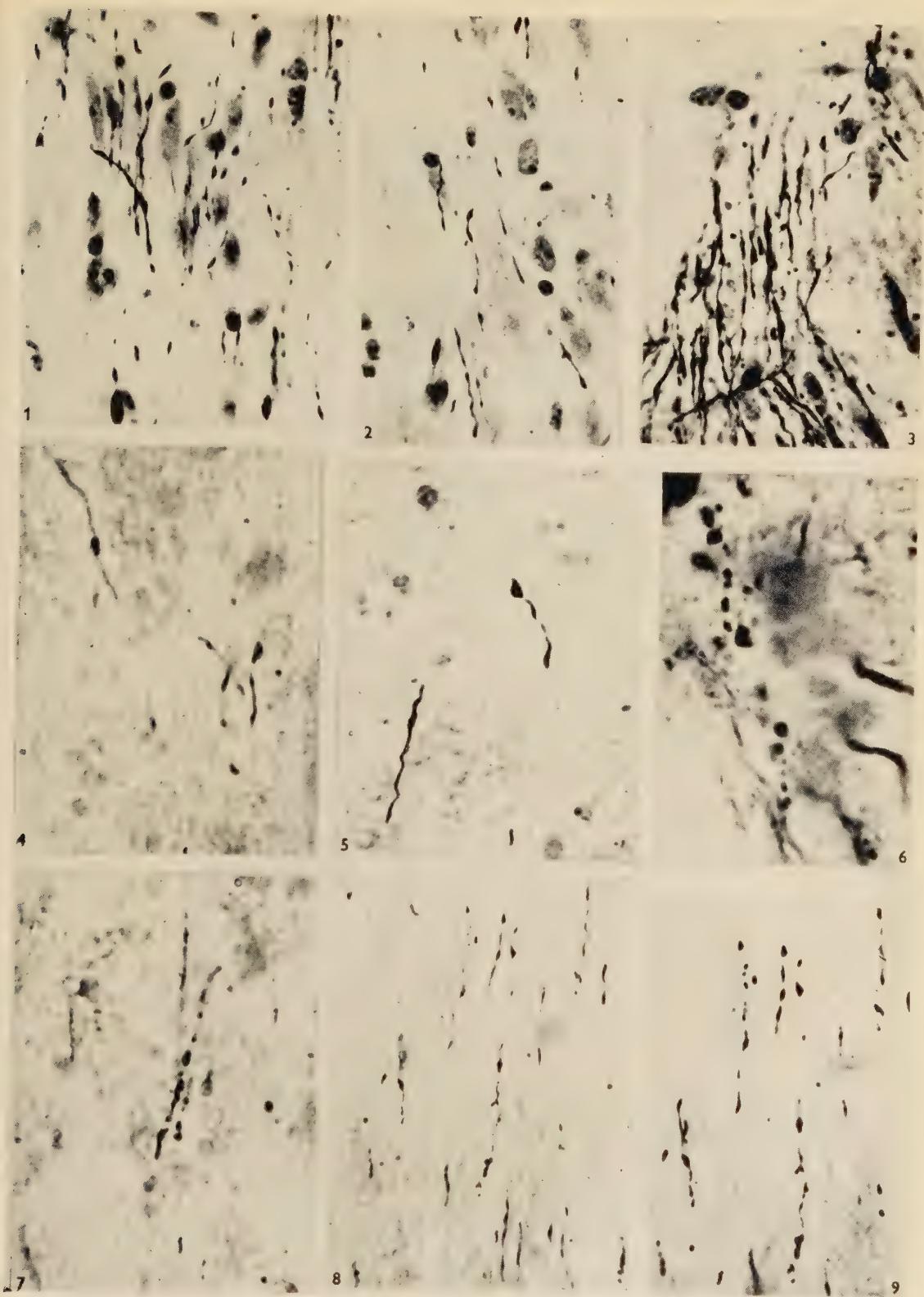
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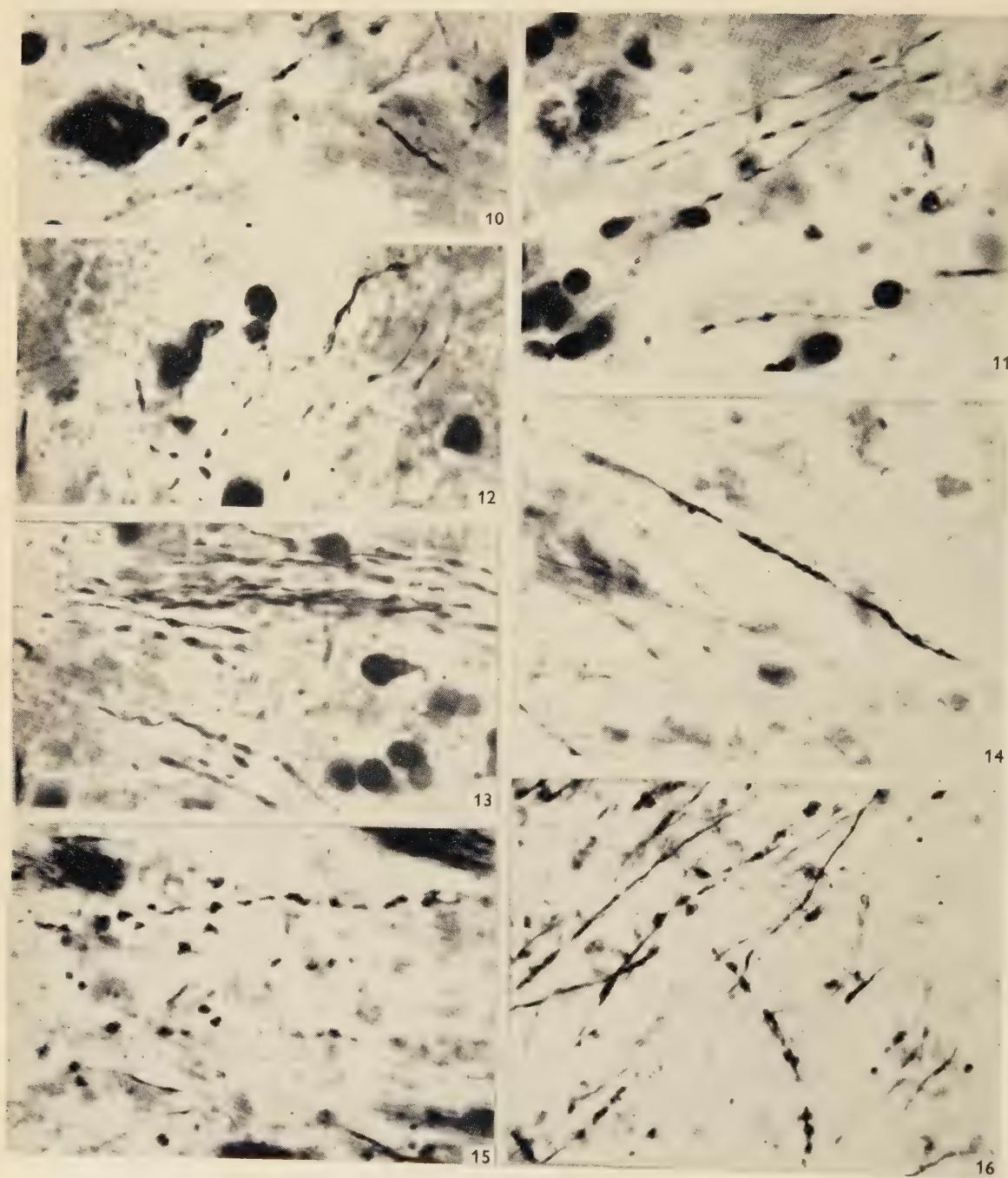
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EXPLANATION OF PLATES

PLATE 1

All figures in this plate are from Rhesus *Am* 1, with an anterior temporal lesion.

Fig. 1. Degenerating fibres at base of septum ipsilateral to lesion. Nauta method. $\times 800$.
Fig. 2. Degenerating fibres at base of septum contralateral to lesion. Nauta method. $\times 800$.
Fig. 3. Degenerating fibres in the anterior commissure contralateral to the temporal lesion. Nauta method. $\times 800$.
Fig. 4. Degenerating fibres in the ipsilateral fornix. Nauta method. $\times 1400$.
Fig. 5. Degenerating fibres in the contralateral fornix. Nauta method. $\times 1400$.
Fig. 6. Droplet terminals in the contralateral ventro-medial hypothalamic nucleus. Glees method. $\times 1800$.
Fig. 7. Degenerating fibres in the ipsilateral bed nucleus of the stria terminalis. Nauta method. $\times 800$.
Fig. 8. Ipsilateral hypothalamic bundle of stria terminalis. Nauta method. $\times 600$.
Fig. 9. Ipsilateral hypothalamic bundle of stria terminalis. Nauta method. $\times 800$.

PLATE 2

Fig. 10. Rhesus *Am* 1. Contralateral intralaminar thalamic nuclei, showing degenerating terminal. Nauta method. $\times 1400$.
Fig. 11. Rhesus *Am* 1. Ipsilateral paramedian thalamic nucleus, with degenerating terminal. Nauta method. $\times 1400$.
Fig. 12. Rhesus *Am* 1. Ipsilateral globus pallidus with numerous swollen terminals. Nauta method. $\times 1200$.
Fig. 13. Rhesus *Am* 1. Ipsilateral globus pallidus, showing degenerating fasciculi traversing the nucleus. Nauta method. $\times 1050$.
Fig. 14. Rhesus *Fr* 1. Degenerating fibre entering ipsilateral dorso-medial hypothalamic nucleus. Glees method. $\times 800$.
Fig. 15. Rhesus *Fr* 1. Degenerating terminals in ipsilateral dorso-medial hypothalamic nucleus. Glees method. $\times 800$.
Fig. 16. Rhesus *Fr* 1. Degenerating terminals in contralateral dorso-medial hypothalamic nucleus. Glees method. $\times 800$.

THE GROWTH OF THE PELVIS IN THE RAT—A MENSURAL AND MORPHOLOGICAL STUDY

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In contrast with the long bones and the skull, the pelvis has rarely been the subject of growth studies. This is surprising in view of the obstetric importance of this part of the skeleton and the theoretical interest which should attach to a structure whose parts, growing essentially like long bones, jointly have to surround an expanding cavity like the skull bones. In this paper four aspects of pelvic growth have been studied, viz. (1) the sites, rates, and amounts of growth in the os innominatum and sacrum; (2) the maturation of the acetabulum; (3) the structure of the sacro-iliac joint; and (4) the development of the sacral intervertebral discs.

The literature relating to these aspects of pelvic growth is scanty and is covered essentially by the work of Strong (1925), Payton (1935), and Bateman (1954), whose observations will be discussed later.

MATERIALS AND METHODS

In all some 131 rats were used.

(1) The sites, rates and amounts of growth were investigated by: (a) taking measurements on serial radiographs of individual animals with or without the use of steel marker-pins; (b) the study and measurement of *autoradiographs* following administration of ^{45}Ca ; and (c) the study of histological sections.

(2) The maturation of the acetabulum was studied: (a) by the examination of cleared alizarin-stained specimens; and (b) by the examination and reconstruction of serial sections.

(3) The structure of the sacro-iliac joint was examined histologically, and the vascular patterns in it were studied by Indian ink and neoprene injections.

(4) The developmental changes in the sacral intervertebral discs were examined histologically.

Measurements

(a) On radiographs, without markers, the following measurements were made:

(1) Length of innominate bone from iliac crest to ischial tuberosity.

(2) Length of the pre-caudal vertebral column from the base of the skull to the sacro-coccygeal intervertebral disc.

(3) Length of ilium.

(4) Length of ischium.

(5) Internal pelvic breadth opposite the ilio-ischial junction.

(6) Length of sacrum in the mid-line.

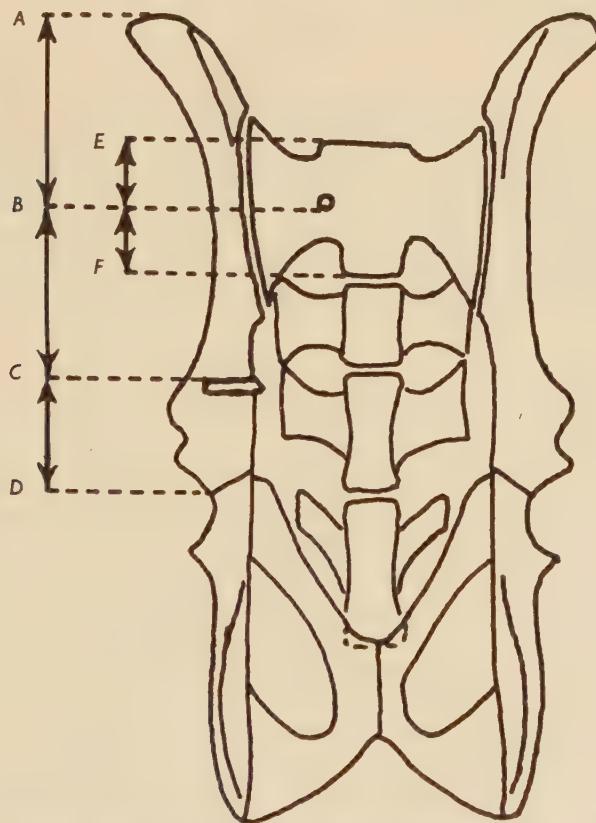
(7) Breadth of sacrum at the level of the mid-point of the sacro-iliac joints.

(b) On radiographs, with markers inserted in the ilium between the sacro-iliac joint and the acetabulum, and in the middle of the first sacral body, the following distances were measured (Text-fig. 1):

- (1) Level of sacral marker to level of iliac crest (*BA*).
- (2) Level of sacral marker to level of ilio-ischial junction (*BD*).
- (3) Iliac marker to iliac crest (*CA*).
- (4) Iliac marker to ilio-ischial junction (*CD*).
- (5) Level of sacral marker to level of iliac marker (*BC*).
- (6) Level of sacral marker to level of upper edge of first sacral body (*BE*).
- (7) Level of sacral marker to level of lower edge of first sacral body (*BF*).

(c) On autoradiographs, measurements were made:

- (1) Of growth increments at the cristal and acetabular ends of the ilium.
- (2) Of growth increments at the upper and lower margins of each sacral body.



Text-fig. 1. A tracing of the radiograph of a rat's pelvis to illustrate the measurements made in animals in which markers had been placed both in the right ilium and in the first sacral body. $\times 2.5$.

Radiographic procedure (forty-one animals)

The 10-day-old animals had their ears marked so that each could be identified during subsequent growth. Radiography was carried out on the tenth day, and at 10-day intervals until 40 days of age, at 20-day intervals until 100 days, and at the 140th, 200th and 365th days of life. On each occasion the animal was anaesthetized with ether, placed on its back on X-ray film, and fixed in a standard position. The X-ray tube was centred above the pelvis at a distance of 60 in. It was calculated, and also proved by comparison of radiographic with direct measurements, that no significant enlargement or distortion of the bony pelvis was produced at this distance, so that measurements could be made directly from the radiographs. This method was limited to the study of growth in the coronal plane, but it had the advantage over the statistical studies on post-mortem specimens that growth could be followed indefinitely in an individual animal. When the animal was 1 year old it was killed, fixed in formalin, and a general post-mortem examination carried out to ensure that it was in a healthy condition.

Insertion of steel markers (four animals)

Under ether anaesthesia an incision was made over the lateral aspect of the flank extending caudally from the ventral end of the iliac crest. The anterior edge of the tensor fasciae latae was identified and retracted dorsally. The ilium was exposed between the sacro-iliac joint and acetabulum in the cleft between the iliacus medially and the glutei dorsally. A small steel pin was then inserted into the bone from its lateral aspect, and the skin incision closed. Through a further incision over the dorsal aspect of the sacrum a similar marker was inserted through the first sacral body.

Autoradiographic procedure (thirty-eight animals)

The technique of Tomlin, Henry & Kon (1953) was closely followed. Calcium carbonate containing radioactive calcium (^{45}Ca : half-life 180 days) was incorporated in the diet. Radioactive feeding continued for a varying period in different rats, no animal receiving a total dose of more than $50\ \mu\text{c}.$, the average being approximately $10\ \mu\text{c}.$ Alternatively, some rats had a single dose of $30\ \mu\text{c}.$ of ^{45}Ca , as calcium chloride, intraperitoneally. Then a non-radioactive diet of calf cubes and green vegetables *ad lib.* was given for varying lengths of time, after which the rats were killed, the pelvis cleaned of soft parts, dehydrated, and embedded in paraffin wax. Blocks were made and rubbed on xylol-covered glass paper until one face of the block showed the required section of the region. An autoradiograph of this surface was made by placing the block in contact with fine grain X-ray film (Ilfex) for a period varying from 48 to 96 hr. The film was then developed and fixed according to a standardized procedure. The bone laid down during radioactive feeding gave a black image on the autoradiograph, while that laid down after cessation of such feeding appeared grey. Measurements of the amounts of new bone added during the known period of non-radioactive feeding could thus be made from the autoradiographs.

Alizarin-staining technique (twenty-one animals)

Before staining with alizarin the pelvis was cleaned of soft parts and the femora removed. It was then fixed in absolute alcohol for at least a week, cleared in 5% aqueous potassium hydroxide for 1-3 days, and stained as a whole preparation by immersion in 1:50,000 sodium alizarin sulphonate in 1% potassium hydroxide until the bones were a deep pink colour. After treatment with 1% potassium hydroxide for 1-2 days to remove excess stain, the specimen was passed through 30%, and 50% glycerine (3 days each), and then into pure glycerine, in which it was stored. The finished preparations were examined under glycerine with a binocular dissecting microscope.

Indian ink injection technique (four animals)

The animals were killed with chloroform and immediately afterwards perfused through the aorta with a mixture of 1 part of Indian ink with 3 parts of distilled water at a pressure of 3 ft. of water for 4-6 hr. After fixation and decalcification, histological sections of the sacro-iliac joints and lumbo-sacral vertebrae were cut from L.V.N.-embedded material.

Histological technique (twenty-three animals)

After fixation in Bouin's fluid, the material was decalcified in 5% trichloroacetic acid and impregnated with paraffin wax by a double-embedding technique which involved the use of 1% celloidin, in order to reduce shrinkage of the tissues to a minimum. Weigert's iron haematoxylin and van Gieson was found to be the most suitable stain, although Mallory's or Masson's trichrome stains, and Harris's haematoxylin and eosin were also used.

OBSERVATIONS

(1) *Growth of the os innominatum*

(a) *Measurements on serial radiographs of individual animals*

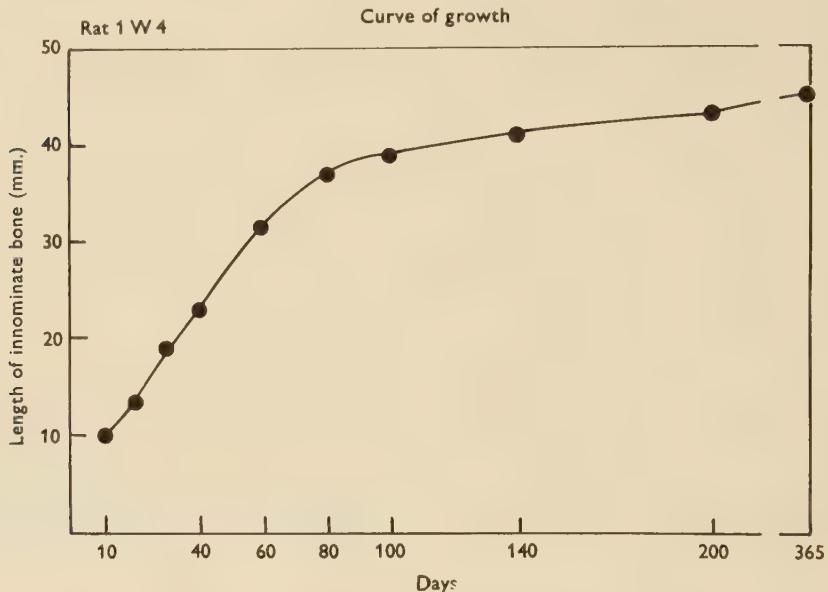
When the length of the innominate bone was plotted against time it was found that growth was fairly rapid until about 80 days of age and then quickly slowed down to a fraction of its former rate, although the bone never completely ceased to grow (at least up to 1 year). In a typical specimen the average daily growth increment fell from 0.55 mm. between 20 and 30 days to 0.05 mm. between 100 and 140 days (Text-fig. 2).

On plotting the length of the innominate bone against the length of the pre-caudal vertebral column close approximation to a straight line was obtained. In a typical specimen the innominate bone length remained a constant fraction ($\frac{5}{16}$) of the vertebral column length between 17 and 248 days of age (Text-fig. 3).

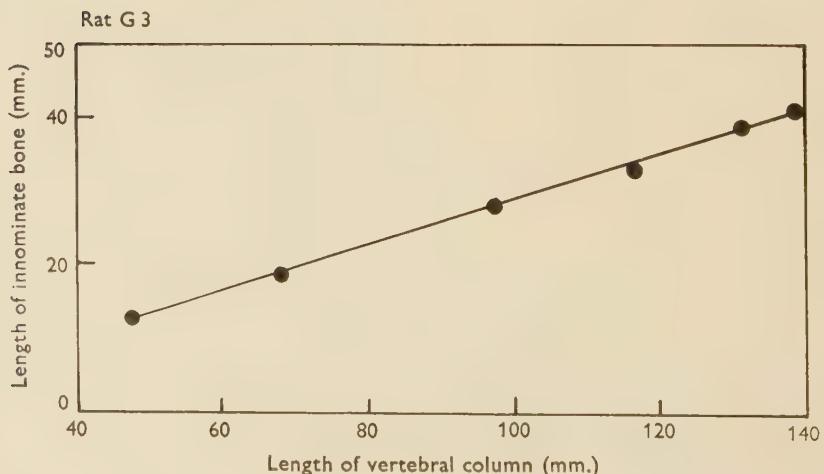
When internal pelvic breadth was plotted against innominate bone length the points again approximated fairly closely to a straight line. In a typical example there was a constant ratio ($\frac{1}{4}$) between the two measurements from the 20th to the 365th day (Text-fig. 4).

The curves of absolute growth for the lengths of the ilium and ischium were

similar in general appearance to that of the innominate bone. Each of the bones grew relatively rapidly until about 80 days and then slowed down. When in a given animal ischial and ilial lengths were plotted against each other, the points were found to lie approximately on a straight line, and calculation showed that during the first year of life the ratio of the length of the ischium to that of the ilium did not depart significantly from 7:10 (Text-fig. 5).

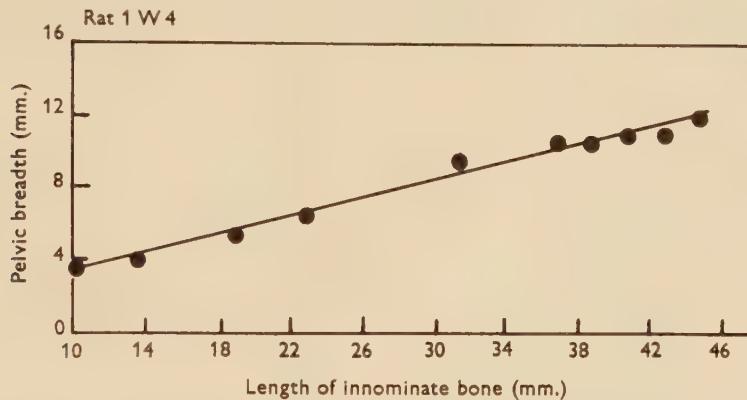


Text-fig. 2. Length of innominate bone plotted against time. (Similar curves were obtained from measurements of forty other rats.)

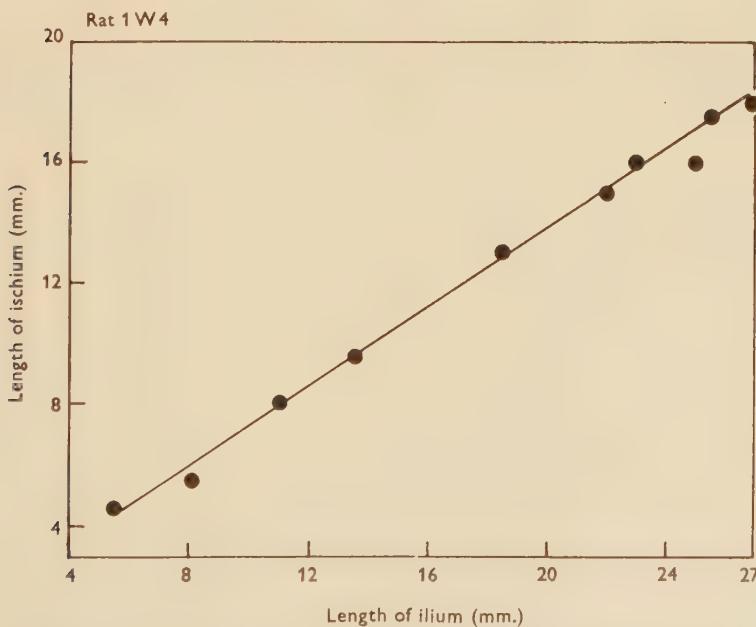


Text-fig. 3. Length of the innominate bone plotted against the length of the pre-caudal vertebral column for rat G3. Three other rats gave similar graphs.

When the curves for the growth of the whole ilium, and for the parts above and below a marker placed in the bone were compared it was found that (a) growth at the crest end was much in excess of growth at the acetabular end, and (b) growth at the acetabular end ceased after 60 days, whereas the crest end was still growing slowly at the end of the first year. The acetabular end of the ilium, between the 20th and 365th days, grew only 2 mm. while the crest end grew 15·7 mm., i.e. over seven times as much. The growth increments in millimetres per day at the two ends, however, were very different at different stages of growth (Text-fig. 6).

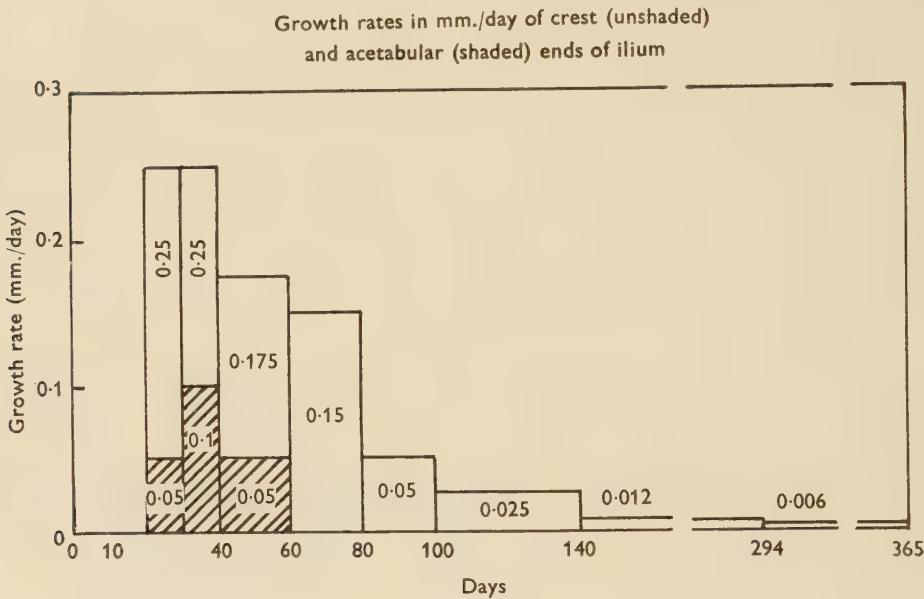


Text-fig. 4. A typical result of plotting pelvic breadth against innominate bone length.
(Plots of the measurements of forty other rats gave similar results.)

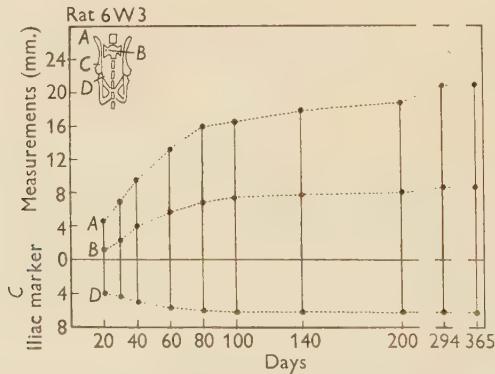


Text-fig. 5. Length of ischium plotted against length of ilium in rat 1W4.
Typical of the results from measurements of forty other rats.

Growth studies of the pelvis, where markers had been placed in both the ilium and the first piece of the sacrum, revealed a striking change in the relations of the two bones during the growth period. With the iliac pin as point of reference (Text-fig. 7) there was an upward (cranial) movement of the sacral pin during growth and this appeared to be related to the upward movement of the iliac crest. If, on the other hand, the points on the ilium were measured from the sacral marker as point of



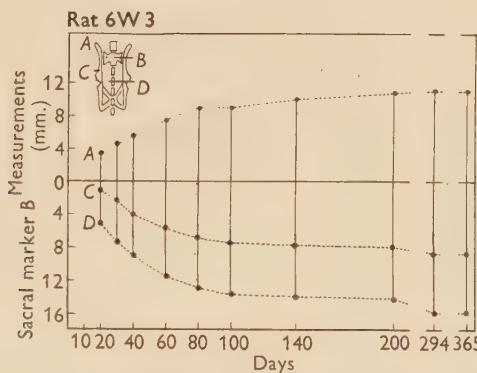
Text-fig. 6. Histogram showing the growth rates in mm. per day of crest (unshaded) and acetabular (shaded) ends of the ilium from the 20th day to the end of the first year.



Text-fig. 7. Growth curves for a rat's pelvis in which markers had been placed at operation in both the right ilium and the first piece of the sacrum. The iliac pin is taken as the reference point for measurements. The upper curve shows the vertical distance of the crest above the iliac pin at different ages; the second curve gives the height of the sacral pin above the iliac pin; while the lower curve gives the distance of the acetabular extremity of the ilium (ilio-ischial synchondrosis) below the iliac pin.

reference (Text-fig. 8) then it was found that the iliac pin moved steadily downwards (caudally) with age, indicating that the ilium as a whole had moved caudally relative to the sacrum. This was simply explained by accretional growth at the crest more than compensating for the downward displacement of the ilium as a whole. In fact, nearly one-half of the cristal increment must have slid caudally past the sacrum during growth. Moreover, during the first 5 months the rate of descent relative to the rate of cristal growth was constant, though the ratio fell a little after that.

It was also found that the ratio of the distances of the sacral marker (at the mid-point of the sacro-iliac joint) from (1) the iliac crest, and (2) the ilio-ischial junction remained almost constant at 11:16 despite the unequal growth at the two ends of the ilium.

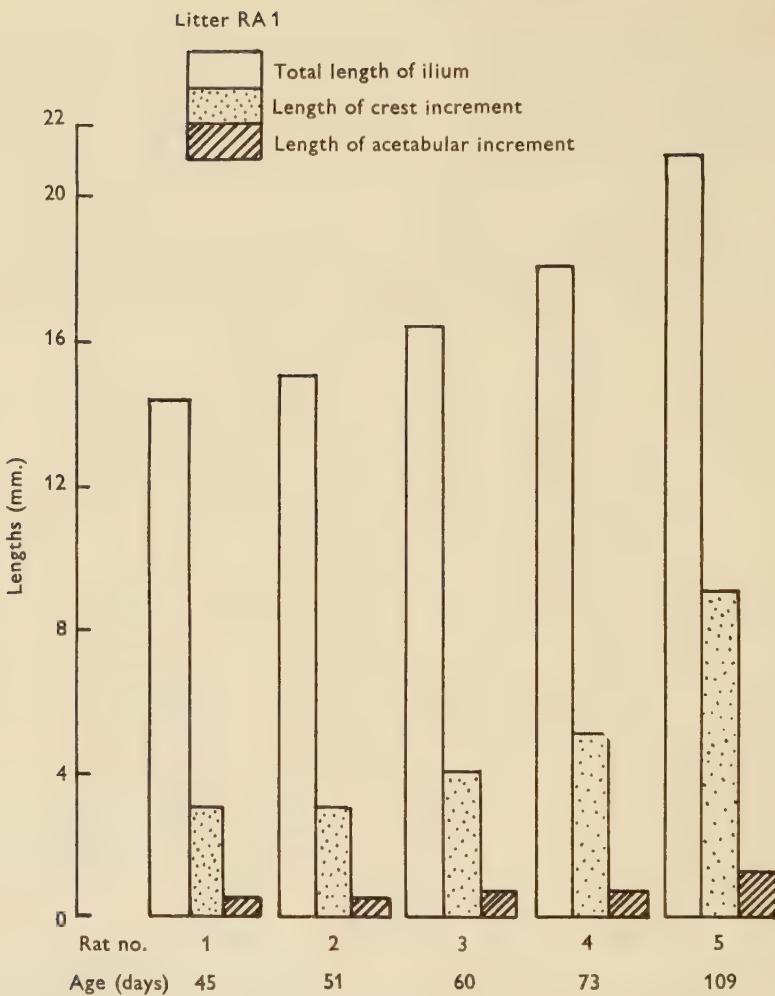


Text-fig. 8. Growth curves for the same pelvis as in Text-fig. 7, but with the sacral pin taken as point of reference for measurements. Note that the iliac pin (C) moves caudally relative to the sacrum with age.

(b) *Study and measurement of autoradiographs*

Study of autoradiographs of animals fed temporarily on a diet containing radioactive calcium followed by a non-radioactive diet (Pl. 1, fig. 3) confirmed that more growth took place at the crest end than at the acetabular end of the ilium. Study of a series of five litter-mates fed with a diet containing ^{45}Ca for 30 days and killed at various ages showed that, after radioactive feeding ceased, six to seven times more growth took place at the crest end than at the acetabular end of the bone (Text-fig. 9).

Autoradiographs of animals killed several weeks after the cessation of radioactive feeding (Pl. 1, fig. 3) also showed that the radioactive part of the ilium did not now extend above the upper margin of the sacro-iliac joint, although the whole ilium right up to the crest, and therefore well above the level of the sacrum, must have been radioactive at the time of cessation of radioactive feeding, for autoradiographs of animals killed soon after the cessation of radioactive feeding showed the whole skeleton to be active. Thus the ilium must have moved caudally relative to the sacrum during growth, which agrees with the conclusions from marker experiments described above.



Text-fig. 9. Histogram constructed from measurements made on the autoradiographs of coronal sections of the pelves of five litter-mates which were given radioactive calcium during the first 30 days of life. The bottom row of figures gives the ages of the animals when killed.

(c) Study of histological sections

Histological sections of the innominate bone showed active growth cartilage and endochondral bone formation along the iliac crest, at the ischial tuberosity, at the symphysis pubis, and at the tip of the inferior ventral spine of the ilium, throughout the first year. Some cartilage was still present in these situations at $3\frac{1}{2}$ years, but it appeared to be inactive in so far as growth was concerned. Growth cartilages were also present in the acetabular region at the extremities of the ilium, ischium and pubis, but these disappeared between 100 and 120 days after birth. Secondary centres of ossification were constantly present only in the acetabulum, but very occasionally small flecks of bone were to be found in the crest cartilage of old animals.

No secondary centres were found at the ischial tuberosity, symphysis pubis, or inferior ventral spine. The cartilages and secondary centres in the acetabular region will be described in detail later.

(2) Growth of the sacrum

(a) Measurements on serial radiographs of individual animals

A. *Length of sacrum relative to length of pre-caudal vertebral column.* Measurements made from the serial radiographs of two series of litter-mates (Tables 1 and 2) showed that in each animal the length of the sacrum remained approximately 18% of the length of the vertebral column (excluding the tail), throughout the period of growth studied.

Table 1

(Measurements in mm.)

Age in days ...	17	35	67	105	173	248
Rat G1						
C1-L6 length	—	56.5	81.0	95.5	109.0	113.0
Sacral length	—	12.5	18.5	21.0	24.0	25.2
Sacral length as a %*	—	(18.1)	(18.6)	(18.0)	(18.0)	(18.2)
Rat G2						
C1-L6 length	42.5	58.5	75.5	91.0	104.0	109.0
Sacral length	9.0	13.0	17.5	19.5	22.5	23.5
Sacral length as a %*	(17.5)	(18.3)	(18.9)	(17.7)	(17.8)	(17.7)
Rat G3						
C1-L6 length	38.5	55.5	80.0	95.5	107.5	113.5
Sacral length	8.5	12.5	17.5	21.0	24.0	25.0
Sacral length as a %*	(18.2)	(18.3)	(18.0)	(18.3)	(18.3)	(18.2)
Rat G4						
C1-L6 length	39.5	58.0	82.5	97.0	105.5	111.0
Sacral length	8.5	12.5	18.0	21.0	23.0	24.5
Sacral length as a %*	(17.5)	(17.7)	(18.0)	(17.8)	(17.9)	(18.3)

* Percentage of the pre-caudal vertebral column.

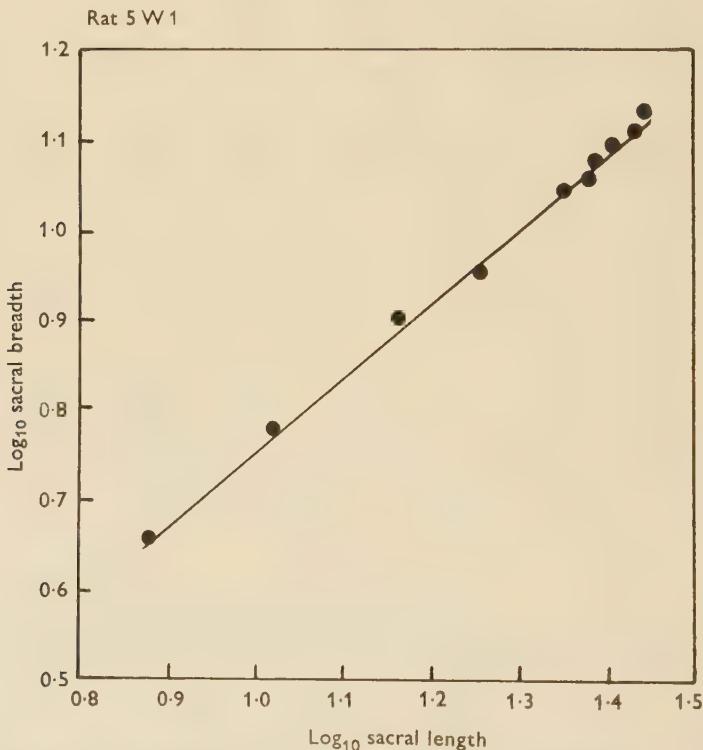
Table 2

(Measurements in mm.)

Age in days ...	14	28	132	205	292
Rat WS1/1					
C1-L6 length	44.0	68.0	111.0	123.0	—
Sacral length	9.5	16.0	26.0	28.0	—
Sacral length as a % of the total length of the pre-caudal vertebral column	(17.5)	(19.0)	(19.7)	(18.5)	—
Rat WS1/2					
C1-L6 length	42.5	63.5	102.0	108.5	111.0
Sacral length	9.3	14.5	23.0	24.5	25.0
Sacral length as a % of the total length of the pre-caudal vertebral column	(17.9)	(18.6)	(18.4)	(18.4)	(18.3)
Rat WS1/3					
C1-L6 length	38.0	64.0	105.0	111.5	111.5
Sacral length	8.5	14.5	23.5	24.5	24.5
Sacral length as a % of the total length of the pre-caudal vertebral column	(18.3)	(18.4)	(18.3)	(18.1)	(18.1)

B. *Length and breadth of the sacrum during growth.* Curves of absolute growth for the length and breadth of the sacrum in individual animals were similar in general appearance to those of the innominate bone. Growth in each dimension was again fairly rapid until about 80 days of age, and then quickly slowed down, although some growth was still taking place at the end of one year.

C. *Breadth of sacrum relative to length of sacrum during growth.* When the logarithm of sacral breadth was plotted against the logarithm of sacral length in a given animal the points lay approximately on a straight line (Text-fig. 10) which indicated that the allometric formula $y = bx^k$ was applicable (where y = sacral breadth, x = sacral length, b is a constant, and k is the measure of relative growth rate). In twenty animals the k values varied between 0.75 and 0.9, indicating bradyauxesis of sacral breadth relative to length, i.e. sacral breadth increased at a constantly slower rate than sacral length.



Text-fig. 10. Plot of the logarithm of sacral breadth against the logarithm of sacral length for rat 5W1. The slope (k) of the line joining the points (i.e. the measure of relative growth rate) is in this case 0.83.

D. *Growth of individual sacral bodies.* Measurement of the growth increments at the cranial and caudal surfaces of the first sacral body when a marker had been placed in this body near the middle line showed that, from the 30th to the 294th day, between two and three times more bone had been added at the caudal surface than at the cranial surface. This was confirmed by autoradiographic studies (Pl. 1, figs. 3, 4).

(b) Study and measurement of autoradiographs

Growth of the sacrum in the antero-posterior direction was difficult to measure from the radiographs in the steel marker experiments because of the smallness of the growth increment, and autoradiographs did not make the position any clearer. Autoradiographs of coronal sections (Pl. 1, fig. 3), however, showed clearly that an even layer of non-radioactive bone had been laid down at the lateral end of the sacral ala beneath the sacro-iliac joint.

(c) Study of histological sections

At 17 days after birth there were in each sacral segment a pair of vertical cartilaginous plates separating the centre of ossification in the centrum from the centres of ossification in the roots of the neural arches (Pl. 2, fig. 10). The appearances were those of a pair of typical secondary synchondroses, the cartilage plates consisting of a central hyaline zone with growth cartilages on either side. Adjacent sacral bodies were separated by an intervertebral disc of cartilage, which likewise consisted of a hyaline zone bounded on its upper and lower surfaces by a layer of growth cartilage (Pl. 2, fig. 13). The fate of these atypical intervertebral discs will be described in detail later. There was also a very active plate of growth cartilage in the ala just beneath the sacro-iliac joint surface.

The neuro-central synchondroses were still present in the first sacral vertebra at 3 weeks, but those in the lower sacral vertebrae had disappeared by synostosis at this time (Pl. 2, fig. 11). During the fourth week those of the first sacral vertebra also synostosed, and thereafter lateral growth of the first piece of the sacrum could only take place at the persistent growth cartilages at the outer extremities of the alae (Pl. 2, fig. 12).

The growth cartilages at the cranial and caudal surfaces of the sacral bodies, and those at the outer ends of the alae, were still histologically demonstrable at $3\frac{1}{2}$ years of age, although they appeared to be inactive.

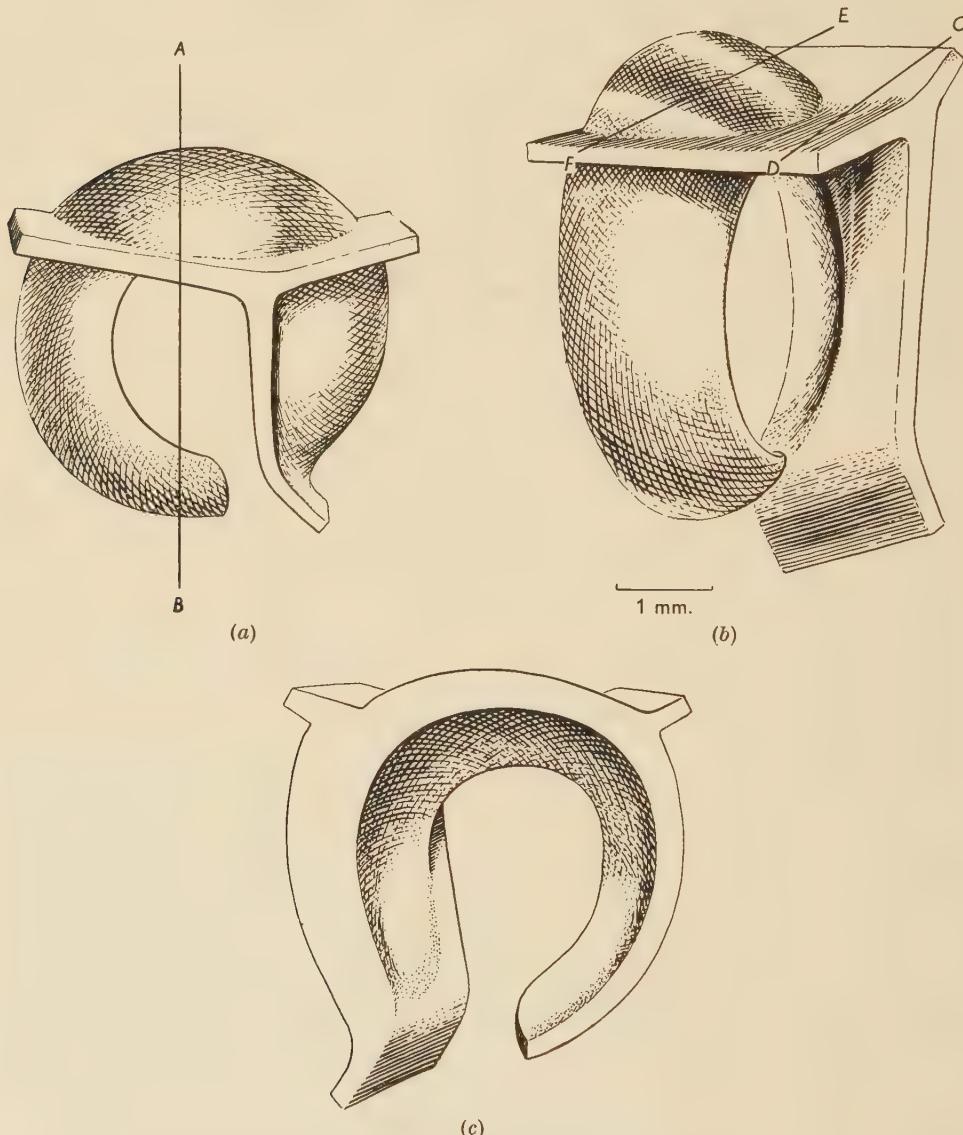
That more growth occurred at the lower than at the upper surface of the vertebral bodies was supported by the direction of the nutrient vessels as they passed through the anterior cortex into the medulla (Pl. 1, figs. 6, 7). Indeed, the over-all shape of each sacral body on mid-line sagittal section, in itself suggested that more growth had taken place at the lower end than at the upper, for the lower half of the hour-glass-shaped body was about twice as long as the upper half (Pl. 1, fig. 6). The emergence of veins at right angles to the posterior surface of the body (Pl. 1, fig. 7), however, suggested that little appositional growth and remodelling occurred on this surface, for it is now well established that the obliquity of nutrient vascular channels is due to the deposition of successive layers of bone beneath that part of the periosteum which is constantly being pulled over the cortex towards the 'growing' end.

*Observations**Maturation of the acetabulum*

It is not possible to give an intelligible or useful account of the development of the acetabular region unless it is appreciated (1) that a distinction must be made between the cartilage of the acetabular socket proper, and the cartilage which intervenes

between ilium, ischium, and pubis, medial to the socket; and (2) that in this region there are three different kinds of cartilage with different functions.

Dissections of the acetabular cartilage during the growth period showed that it had a medial tri-flanged part continuous laterally with a cup-shaped part (Text-fig. 11 *a-c*). The medial part of the cartilage intervened between the ilium, ischium, and pubis, while the lateral part helped to form the acetabular socket. When the

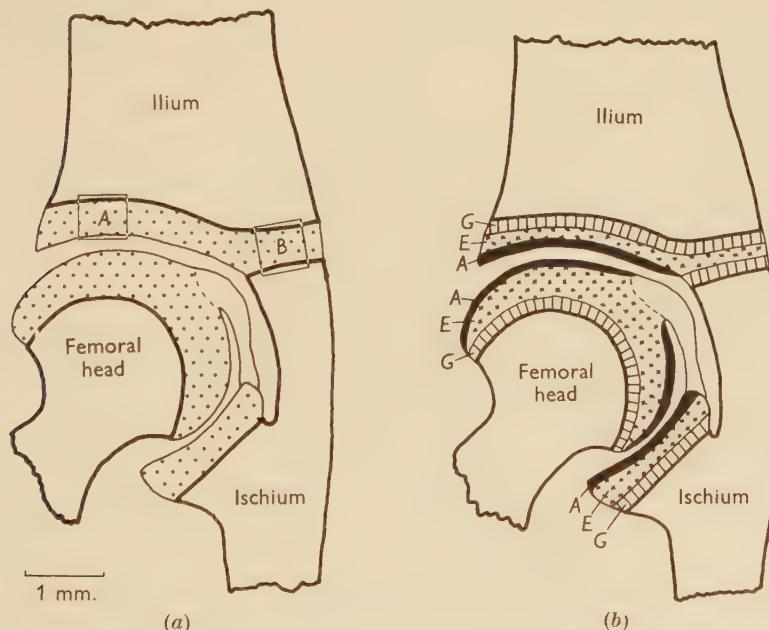


Text-fig. 11. Drawing of the acetabular cartilage complex dissected from a 40-day-old rat. (a) Medial view; (b) postero-lateral view; and (c) lateral view. The line *AB* in (a) represents the plane of section in Text-fig. 12 *a* and *b*; and the lines *CD* and *EF* in (b) represent the planes of section in Text-fig. 13 *a* and *c* respectively. (a) and (c) are approx. $\frac{5}{6}$ scale of (b).

cartilage was observed from the lateral aspect only the cup-shaped part was to be seen, the tri-flanged medial part being hidden from view.

A coronal section through the middle of the acetabulum (Text-fig. 12a) showed that its upper and lower walls were formed by the cup-shaped lateral part of the cartilage, while its medial wall was bounded by non-articular ischium. Further, the cartilage in the upper wall was directly continuous medially with a horizontal flange of the medial part.

Histological examination of the lateral part of the acetabular cartilage (Pl. 3, fig. 14) forming the walls of the hip socket showed that it could be divided into three layers, each differing in structure. First there was a thin band of *articular cartilage* lining the joint cavity where the cells were flattened and the matrix was coarsely fibrous and stained heavily with van Gieson. Outside this there was a zone of *hyaline cartilage* with rounded cells separated by clear matrix, and, finally, there was a thick zone of pale *growth cartilage*, with cells arranged in columns, lying adjacent to the bony metaphyses. It is proposed to call the middle zone epiphyseal cartilage because it was in this layer that secondary centres appeared, and because it is homologous with the epiphyseal cartilage masses at the ends of the long bones. Each flange of the tri-flanged medial part of the acetabular cartilage (Pl. 3, fig. 15) was composed of two layers of growth cartilage separated by a layer of hyaline cartilage, similar in appearance to, and continuous with, the epiphyseal cartilage in the lateral cup-shaped part (Text-fig. 12b).

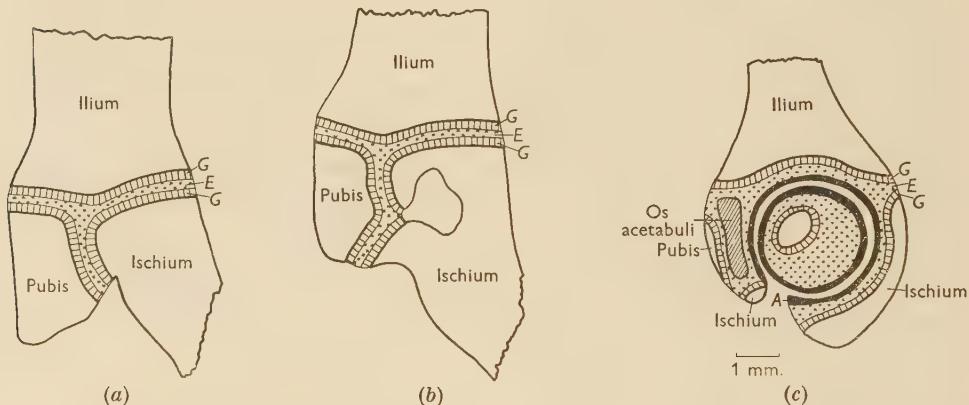


Text-fig. 12. (a) A coronal section through the acetabulum of a 52-day-old rat (semi-diagrammatic).

The acetabular cartilage and the cartilage of the femoral head is stippled. A photomicrograph of area A is shown in Pl. 3, fig. 14, and of area B in Pl. 3, fig. 15. (b) The distribution of the three types of cartilage in the acetabular region: (A) articular, (E) epiphyseal, and (G) growth cartilage.

Sagittal sections (Text-fig. 13 *a-c*) through the acetabular cartilage showed (1) the typical triradiate appearance of the medial part; (2) that the floor or medial wall of the acetabular cavity was formed by the ischium; and (3) that the secondary centres appeared in the epiphyseal cartilage of the lateral cup-shaped part. This cartilage was thickest opposite the pubis and least thick opposite the ischium.

Examination of alizarin-stained whole specimens showed that calcification of the epiphyseal cartilage began opposite the pubis on the 24th day and a separate area of calcification appeared opposite the ilium on the 35th day. Calcification then spread from the latter area throughout the remainder of the epiphyseal cartilage of the acetabular cup, joining the area of calcification opposite the pubis and extending backwards into the cartilage opposite the ischium. Secondary centres, one for the *os acetabuli* and one for the acetabular epiphysis, appeared in this calcified epiphyseal cartilage.



Text-fig. 13. Sagittal sections of the acetabular region of a 52-day-old rat (semi-diagrammatic) to show the distribution of the three types of cartilage and the position of the *os acetabuli*. (a) Medial to the socket—plane *CD* in Text-fig. 11*b*. (c) Through the middle of the socket—plane *EF* in Text-fig. 11*b*. (b) In a plane parallel to but between those of (a) and (c).

The centre for the *os acetabuli* appeared 28 days after birth in the thick epiphyseal cartilage adjacent to the lateral end of the pubis. At 90 days the centre had enlarged into a box-shaped bony element, 0.5 mm. thick, which had almost entirely replaced the epiphyseal cartilage opposite the pubis. Its broad antero-medial surface lay on the pubic metaphysis (Pl. 2, fig. 9); its acetabular (postero-lateral) surface was covered with articular cartilage; its antero-lateral surface was superficial and covered with periosteum; its postero-medial face lay on the ischium; its narrow superior face was related to the iliac metaphysis (Pl. 3, fig. 18); and its inferior face abutted on the ischium.

The centre for the *acetabular epiphysis* appeared between 70 and 80 days after birth in the epiphyseal cartilage adjoining the ilium, although calcification was present in this site as early as 35 days. The acetabular epiphysis was thinner and smaller than the *os acetabuli*, and was virtually only a small scale covering the iliac metaphysis. It began to ossify in the thicker lateral part of the epiphyseal cartilage opposite the ilium (Pl. 2, fig. 8) and spread anteriorly and posteriorly, meeting the

os acetabuli at 90 days and fusing with it. The combined epiphysis then joined the ilium, pubis, and ischium soon after 100 days when the three bones synostosed. Small isolated areas of cartilage were still to be found, however, along the margins of the junction of those bones up to 170 days.

The situation and maturation behaviour of the os acetabuli and acetabular epiphysis made it clear that they were in reality secondary ossifications for the pubis and ilium respectively.

The structure of the sacro-iliac joint during growth

It has already been shown, from marker-pin experiments and by autoradiography, that during growth the ilium migrates caudally relative to the sacrum. As it seemed probable that the migration occurred at the joint between the two bones, this was examined histologically in rats at different ages in the hope of throwing light on the mechanism of descent.

Coronal sections of the sacro-iliac joint during the growing period (Pl. 3, fig. 19) showed that the joint was diarthrodial inferiorly and syndesmodial superiorly. In the diarthrodial part a thick layer of cartilage covered the sacrum but the opposed surface of the ilium was covered only by a thin layer of fibrous tissue. The syndesmosis displayed numerous bands of fibrous tissue running with various degrees of obliquity between sacrum and ilium. The uppermost of these bands (hereafter called the superior sacro-iliac ligament) was the longest and strongest, and passed from the supero-lateral border of the sacral ala to the growth cartilage at the iliac crest: therefore, in coronal section, the sacrum appeared to be slung from the iliac crests by a pair of suspensory ligaments (Pl. 1, fig. 1). The lower bands, passing between the sacrum and the ilium, were not nearly so dense and were separated from one another by thin-walled vascular channels, which when studied by neoprene or Indian ink injection, were seen to be venous sinuses (Pl. 1, fig. 2). This arrangement of fibre bundles interlaced with blood spaces was very reminiscent of the structure of a cranial suture (Pritchard, Scott & Grgis, 1956).

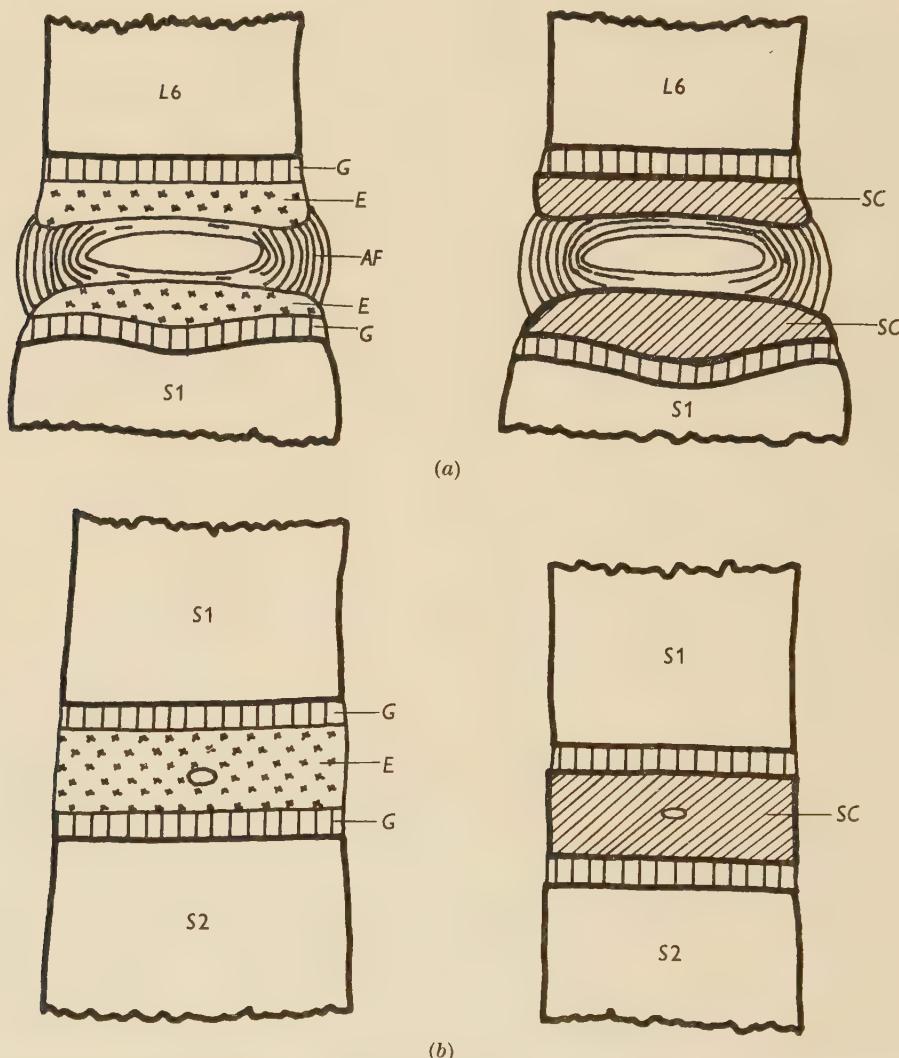
The medial surface of the ilium above and below the syndesmodial part of the joint was smooth, but while the lower diarthrodial surface was covered only by the thin layer of tangential fibres already mentioned, the surface *above* the syndesmosis was cushioned by a remarkably thick periosteal layer in which the fibre bundles were predominantly radial, projecting from the bone in a series of tufts. At the syndesmosis itself, however, the ilium was roughened and pitted by numerous erosion bays, in many of which osteoclasts could be seen (Pl. 1, fig. 5).

Transverse sections through the pelvis just above the level of the sacro-iliac joint (Pl. 3, fig. 17) showed the thick periosteal cushion on the medial surface of the ilium contrasting with the thin layer of periosteum on the lateral surface. The strong superior sacro-iliac ligament was also evident along the inner margin of the thickened periosteum.

The subdivision of the sacro-iliac joint into lower diarthrodial and upper synarthrodial parts was still present in old age ($3\frac{1}{2}$ years) as was also the superior sacro-iliac ligament. These observations formed the basis for a hypothesis concerning the mechanisms of iliac descent which will be discussed later.

*Observations**The development of the sacral intervertebral discs*

As mentioned earlier in this paper adjacent sacral bodies at 17 days (Pl. 2, figs. 10, 13) were separated by an intervertebral disc of cartilage, which consisted of a hyaline zone bounded on its upper and lower surfaces by a layer of growth cartilage. The sacral intervertebral synchondroses were quite different in their structure, even at this early stage, from the joints between the bodies of the other vertebrae. There



Text-fig. 14. Diagrams comparing the structure and maturation of a typical intervertebral joint such as that between *L* 6 and *S* 1 (*a*) with that found between the sacral bodies (*b*). On the left the diagrams illustrate the joints *before*, and on the right *after*, the appearance of the secondary centres of ossification. *G*, growth cartilage; *E*, epiphyseal cartilage; *SC*, secondary centre of ossification; *AF*, annulus fibrosus.

was, however, a small nucleus pulposus with its typical cells in the middle of each sacral disc.

During the third week a horizontal ring of ossification appeared around the central nucleus of the sacral intervertebral discs (Pl. 2, fig. 11). Ossification appeared first in the disc between the first and second sacral bodies and then a few days later between the second and third discs and so on. (Because of their ring shape these ossifications were cut twice in coronal sections.)

The bony centres in the discs enlarged so that by $4\frac{1}{2}$ weeks (Pl. 2, fig. 12) there was a bony plate between the first and second sacral bodies, replacing the original hyaline zone of cartilage, and bounded above and below by a layer of growth cartilage. It was also observed at $4\frac{1}{2}$ weeks that a secondary centre was about to form at the caudal end of L6, although no signs of impending ossification were present at the upper end of S1. In fact, for vertebrae other than the sacral, secondary centres always appear at the caudal end of a given vertebral body before the cranial end.

By six months (Pl. 3, fig. 16) the maturation of the sacral region was nearly completed, and well-ossified discs were found between each of the sacral vertebrae, separated, however, by plates of growth cartilage from the vertebral bodies. Such growth cartilages were still present in extreme old age ($3\frac{1}{2}$ years), indicating that the 'disc bones' remained as separate entities throughout life and did not fuse with the sacral vertebral bodies.

The intervertebral discs in the sacral region were therefore quite different from those in other parts of the vertebral column. In the vertebral column generally, apart from the sacrum, in passing from one vertebral body to the next (Text-fig. 14a) there was (a) a layer of growth cartilage, (b) a layer of hyaline epiphyseal cartilage in which a secondary centre later appeared, (c) the annulus fibrosus surrounding the nucleus pulposus, (d) another layer of epiphyseal cartilage like the former, and (e) the growth cartilage of the next body; in the sacral region, however, the annulus fibrosus was absent (Text-fig. 14b), and the nucleus pulposus was very small so that the two epiphyseal cartilage plates of typical discs were represented by a single fused plate. This then ossified from a single centre giving rise to a single disc bone in place of the paired secondary centres of typical discs. Neither the single disc bones of the sacrum, nor the paired disc epiphyses of the rest of the vertebral column, however, ever fused with the bodies, for the growth cartilages persisted indefinitely.

DISCUSSION

Growth of the os innominatum

It has been shown that the length of the innominate bone as a whole, as well as the length of the ilium and ischium individually, increased fairly rapidly until about 80 days, which is about the time of puberty, and then slowed down, although growth never ceased completely. This finding agrees with that of Donaldson (1919) who showed that the absolute weight of the entire fresh skeleton in the rat follows a similar time course.

The results also showed that the ratios of the length of the innominate bone to (1) the length of the pre-caudal vertebral column, and to (2) the internal pelvic

breadth, remained constant during the period of observation, and also that the lengths of the ischium and ilium maintained a constant ratio to each other. From this it may be inferred that pelvic growth in the rat is not subjected to specialized local control, but is regulated by general growth determinants.

The marker experiments using radioactive calcium and steel pins showed that the crest end of the ilium grew approximately seven times faster than the acetabular end, and, further, that the acetabular end ceased to grow at about 60 days of age, although the crest was still actively growing at that time. This is similar to the growth pattern of a long bone where one end grows faster than the other, and the faster growing end is usually the last to stop growing. At first sight it might be anticipated that such a marked inequality of growth at the two ends of the ilium would result in the sacro-iliac joint occupying a relatively lower position in the older animal than in the younger, but in fact the relative position of the joint remained unchanged. It follows from this that the joint must migrate towards the iliac crest during growth, or, what amounts to the same thing, the ilium must migrate downwards past the sacrum. It was shown by the experiments described in this paper that relative to a fixed point on the ilium there was upward movement of the crest, due to the activity of the crest growth cartilage, and also upward movement of the sacro-iliac joint. Relative to a fixed point on the sacrum, however, upward movement of the crest was associated with a downward movement of the ilium as a whole; and the two opposing movements were so balanced that the ratio of the distances of the mid-point of the sacro-iliac joint from the crest and acetabular ends of the ilium remained constant. The older pelvis was therefore a proportionate enlargement of the younger, in spite of disproportionate growth at the two ends of the ilium.

This conclusion that a downward shift of the ilium relative to the sacrum occurs during growth is supported by Payton's (1935) work on the bones of madder-fed pigs, where it was found that the increase in the distance of the ventral edge of the sacro-iliac joint from the iliac crest during growth was much less than the amount of new bone added at the crest in the same period of time. From this Payton concluded that the sacro-iliac joint must migrate up the medial surface of the ilium.

Growth of the sacrum

In view of the well-known crano-caudal growth gradient during development, it was surprising to find that from as early as the 17th day of life, up to maturity, the length of the rat's sacrum was a constant proportion of the length of the pre-caudal vertebral column. Similar results were obtained by Schultz (1940, 1944) in the chimpanzee and gibbon, indicating that in the anthropoid apes also sacral length partakes of the general growth characteristics of the rest of the pre-caudal vertebral column.

Growth in the length and breadth of the sacrum, as in the case of the os innominatum, was fairly rapid up to sexual maturity, but then it slackened, continuing at a slow rate indefinitely. The relative lengthening and narrowing of the sacrum during growth shown by the differential growth curves, suggests that the factors controlling sacral breadth may be different from those controlling growth in length. As has just been indicated, sacral length appears to be in line with the growth of the vertebral column as a whole, implying general non-specific control; sacral breadth, on the

other hand, may well be partly under the influence of local factors, such as weight bearing through the acetabulum. Furthermore, it might be expected that growth in sacral breadth should be correlated rather intimately with lower limb development. That local factors do in fact influence sacral breadth was shown in experiments, to be reported later, where bilateral removal of the iliac crest cartilages led to marked broadening of the sacrum.

Growth in sacral width was shown to be due to the growth cartilages in the alae just deep to the sacro-iliac joint surfaces. The iliac surface opposite the sacro-iliac joint, on the other hand, appeared to be quiescent. These findings were confirmed by the autoradiographs, which showed considerable increment of new bone on the sacral side of the joint, but none on the ilial side. Payton (1935) observed similar inequalities of growth on either side of the sacro-iliac joint in the madder-fed pig. He also reported contributions to increasing sacral breadth from the activities of the neuro-central growth cartilages. These growth sites have already been described and illustrated in the rat, and they must play an active part in broadening the sacrum at an early stage; but, unlike the pig, the neuro-central joints in the rat became synostosed early, and after this the entire onus for growth in width of the sacrum, and hence, apart from accretion on the external surface of the ilium, for the widening of the pelvis, must rest on the activity of the growth cartilages beneath the sacro-iliac joints.

The unequal growth of the sacral bodies (and indeed of all the vertebral bodies) at their cephalic and caudal surfaces, so clearly demonstrated autoradiographically and by steel markers, is a direct contradiction of Bateman's (1954) conclusion from the study of grey-lethal and microphthalmic mice, that growth was approximately equal at the two ends. This discrepancy between my results in the rat and Bateman's in the mouse cannot be explained at present unless there is a species difference. It is not obvious why there should be unequal growth at the two ends of a vertebra, but it may be that there are inherently unequal growth potentials in the cephalic and caudal parts of the sclerotomes, and that these persist.

The histological finding that enlargement of the antero-posterior dimension of the sacral body must be almost entirely due to accretion on the anterior surface, agrees with the observations of Knutsson (1948), using Schmorl's nodes as 'natural' markers, on the growth of the lumbar and thoracic vertebrae of the human spine. This pattern of growth is reasonable when one considers the progressive embarrassment to the spinal cord and cauda equina which would follow continuing accretion on the posterior surface of the bodies, unless complex and entirely hypothetical remodelling of the neural arches were to accompany it.

Maturation of the acetabulum

These studies of normal acetabular maturation in the rat have shown that two secondary centres of ossification appear in the epiphyseal cartilage of the acetabulum, one related to the pubis, and the other related to the ilium. These are bony epiphyses in every way equivalent to those at the ends of long bones. The ossifications are preceded by calcification of the epiphyseal cartilage, and this calcification may be confused with ossification if studies are confined to macroscopic examination of alizarin-stained preparations.

As has been shown, the acetabular region exhibits three kinds of cartilage, namely, growth cartilage, epiphyseal cartilage, and articular cartilage, each with a distinctive histology and function. The term 'triradiate cartilage' is used in the literature as if synonymous with the whole of the non-articular cartilage in the acetabular region, i.e. the whole complex of growth cartilages and epiphyseal cartilage which not only separate the growing bones, but also provide the walls of the acetabulum. It is true that the medial part of this cartilaginous complex, lying in the depth of the acetabulum, is triradiate in form, but until relatively late in maturation the complex includes the extensive lateral mass of cartilage which forms the basis of the acetabular wall, and which is not triradiate but shaped like the developing optic cup. In other words, the acetabular cartilage complex consists of a triradiate 'stem' next to the pelvic cavity, flaring out laterally into a cup-shaped socket. Failure to appreciate this has led most workers to describe events, such as the appearance of secondary centres of ossification, as if they were occurring in the triradiate 'stem' whereas, in fact, such ossifications occur in the 'cup' part of the acetabular cartilage.

Accounts of acetabular development in the literature are often confused, rarely in agreement with one another, and they suffer from vagueness and ambiguity, due to the loose way in which the term 'triradiate cartilage' is employed. Strong (1925), giving the times of appearance of centres of ossification in the rat, states that the os acetabuli appears at one month and the acetabular epiphysis at the end of the second month, but does not discuss their position or relations. The general position and times of appearance of the acetabular secondary centres in other animals have been discussed by Gegenbaur (1876), Krause (1876), Flower (1885), Bryce (1915), Bolk, Göppert, Kallius & Lubosch (1938). More recently, Schmidt (1954), in an article on the radiological appearances of the secondary acetabular centres in man, has called for a revision of the nomenclature. He states that around puberty a secondary centre, which he terms the os acetabuli, appears in the acetabular cartilage, and comments that this name was coined by Krause for the acetabular bone described in 1737 by Albinus. He crystallises the views of nearly all authors on acetabular ossification in two statements, namely, (1) that the ossification of the acetabulum results in the formation of several socket bones, or epiphyses, which appear in relation to the cartilage joint and at the acetabular rim, and (2) that one such bone is found constantly between the ilium and pubis in the ventral region of the acetabular floor, and is generally named the os acetabuli.

It has been shown above that, in the rat, the os acetabuli and acetabular epiphysis are secondary centres of ossification, constant as to position, times of origin and fusion, and in every respect equivalent to other bony epiphyses. Further, they are developed in the epiphyseal cartilage of the roof and front wall of the acetabular 'cup', and not in the medial triradiate 'stem' of the acetabular cartilage.

The structure of the sacro-iliac joint during growth

The histological features of the joint here described not only provide support for, and evidence as to the mechanism of, sacro-iliac joint migration, but also raise problems of general interest. Thus the chief obstacle to downward migration of the ilium should be the tension in the short fibrous bands uniting the bones across the syndesmosis part of the joint. As has been shown, however, it is precisely these

fibres which are being detached from the ilium by osteoclastic erosion of the bony surface. Then again the smooth upper part of the medial surface of the ilium, with its cushioning layer of thickened periosteum, and its numerous interfascicular venous sinuses, is just what would be needed to facilitate the passage of the ilium down past the sacrum. The strong superior sacro-iliac ligaments attached to the iliac crests are evidently the means by which the sacrum is slung along the ilia; but, in addition, the ligament on each side could well be an essential factor in the descent of the ilium through its tension resisting upward extension of the growing crests. This would result in room being found for the new metaphyseal bone forming beneath the crest only by a downward movement of the os innominatum as a whole. Direct experimental support for this suggested role of the sacro-iliac ligament will be presented in a later paper.

The peculiar histological structure of the sacro-iliac joint itself is worthy of comment. The presence of cartilage on the sacral, but not on the ilial, side of the diarthrodial lower part of the joint is adapted to the circumstances that the growing lateral cancellous part of the sacrum is much more in need of protection against pressure stresses than the relatively quiescent compact bone on the iliac face of the joint. The absence of cartilage from both sides of the syndesmodial upper part of the joint suggests that here the sacral surface is under tension from the sacro-iliac ligaments, rather than under pressure from the ilium, for it is well known that bones are only covered with cartilage where they are subjected to moderately severe pressure and shearing stresses.

The development of the sacral intervertebral discs

The unique structure of the sacral intervertebral discs was a surprising finding, yet this and the ossification of the discs to produce independent disc bones is functionally intelligible in view of the restrictions on movement between the bodies in this region. No mention of these specialized discs in the rat is to be found in the literature, nor is there a clear account of the way in which the individual sacral bodies are transformed into the mature synostosed sacrum in other animals. Preliminary studies, not reported in this paper, show that 'disc' bones between the sacral bodies are also found in the guinea-pig, rabbit, and drill (*Papio leucophaeus* C.). Fawcett (1907), in his paper on the later stages of ossification of the human sacrum, gives the current view that union of the sacral bodies is effected 'by fusion of the epiphyseal (bony) plates, a pair of which make their appearance between the centre of each segment', but he gives no histological details. Dawson (1925) found that many epiphyses in the limb bones of the rat failed to unite, and in the present study it was found that the epiphyses of the presacral vertebral bodies also failed to unite with their respective bodies. This general persistence of epiphyses in the rat supports the view that the independent sacral 'disc bones' may also represent the epiphyses of the sacral bodies.

SUMMARY

The following aspects of pelvic skeletal growth have been studied in the rat: the sites, rates, and amounts of growth in the os innominatum and sacrum; the maturation of the acetabulum; the structure of the sacro-iliac joint during growth; and the development of the sacral intervertebral discs.

The methods employed included serial radiography of individual animals; autoradiography following ^{45}Ca administration; examination of gross preparations stained with alizarin; and the study of histological sections.

Growth of the innominate bone and sacrum is rapid up to the time of puberty (80 days) and then slows down, but some growth is still taking place at the end of the first year. The ratios of the length of the innominate bone to (1) the length of the pre-caudal vertebral column, and to (2) the internal pelvic breadth, remain constant between the 17th and 248th days, and also the lengths of the ischium and ilium maintain constant proportions to each other. The cristal end of the ilium grows seven times faster than the acetabular end. Growth at the acetabular end ceases at 60 days, but cristal growth continues at least to the end of the first year. During growth the ilium migrates caudally relative to the sacrum through a distance approximately equal to half the vertical increment of new bone added at the crest metaphysis.

Sacral breadth increases at a slower rate than sacral length. The sacral bodies grow at least twice as fast at their caudal as at their cranial surfaces.

The acetabular cartilage is shown to be triradiate medially and cup-shaped laterally, and to consist of growth, epiphyseal, and articular types of cartilage. The os acetabuli and acetabular epiphysis are secondary centres of ossification, related to the pubis and ilium respectively, which appear in the epiphyseal cartilage of the cup-shaped lateral region.

The structure of the sacro-iliac joint is shown to be consistent with the fact of caudal migration of the ilium relative to the sacrum during growth.

In the sacral region the bodies of the vertebrae are first separated by hyaline cartilage, which later ossifies to form 'disc' bones. It is suggested that these represent the fused epiphyses of adjacent bodies which have failed to unite with the bodies.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A coronal section of a 62-day-old rat pelvis. Weigert's haematoxylin and van Gieson. $\times 4.3$.

Fig. 2. An oblique paramedian section of the sacro-iliac joint in a 5-month-old rat, in which the left ventricle had been injected with Indian ink, to show the vascular arrangement in the syndesmodial part of the joint. Counterstained with van Gieson. $\times 7$.

Fig. 3. An autoradiograph of a coronal section of the pelvis of a 73-day-old rat which had been fed with a diet containing radioactive calcium for the first 30 days of life. $\times 1$.

Fig. 4. An autoradiograph of a median sagittal section of the last two lumbar and first two sacral vertebrae in a 100-day-old rat which had been given a single injection of 30 μ c. of ^{45}Ca (as calcium chloride) when 34 days old. $\times 1$.

Fig. 5. A coronal section of the medial aspect of the ilium at the syndesmodial part of the sacro-iliac joint in a 10-week-old rat showing an osteoclast in an erosion bay. Haematoxylin and eosin. $\times 710$.

Fig. 6. A median sagittal section through the first sacral body of a 10-week-old rat. Weigert's haematoxylin and van Gieson. $\times 10$.

Fig. 7. A sagittal section through the second sacral body in a 4½-month-old rat which had been perfused through the left ventricle with Indian ink. Counterstained with van Gieson. $\times 18$.

PLATE 2

Fig. 8. A coronal section through the superior acetabular rim in a 70-day-old rat. Weigert's haematoxylin and van Gieson. $\times 80$.

Fig. 9. A transverse section through the anterior wall of the acetabulum at 90 days. Weigert's haematoxylin and van Gieson. $\times 26$.

Fig. 10. A coronal section of the sacrum in a 17-day-old rat. Masson. $\times 10$.

Fig. 11. A coronal section through the first piece of the sacrum in a 3-week-old rat. Weigert's haematoxylin and van Gieson. $\times 17$.

Fig. 12. A coronal section through the first piece of the sacrum in a 4½-week-old rat. Weigert's haematoxylin and van Gieson. $\times 12$.

Fig. 13. The area contained in the rectangle in Fig. 10. $\times 47$.

PLATE 3

Fig. 14. A coronal section through the roof of the acetabulum at 52 days (area A in Text-fig. 12a). Weigert's haematoxylin and van Gieson. $\times 110$.

Fig. 15. A coronal section through the cartilage between ilium and ischium medial to the acetabulum at 52 days (area B in Text-fig. 12a). Weigert's haematoxylin and van Gieson. $\times 110$.

Fig. 16. A coronal section through the 'disc' bone between *S3* and *S4* in a 6-month-old rat. Weigert's haematoxylin and van Gieson. $\times 10$.

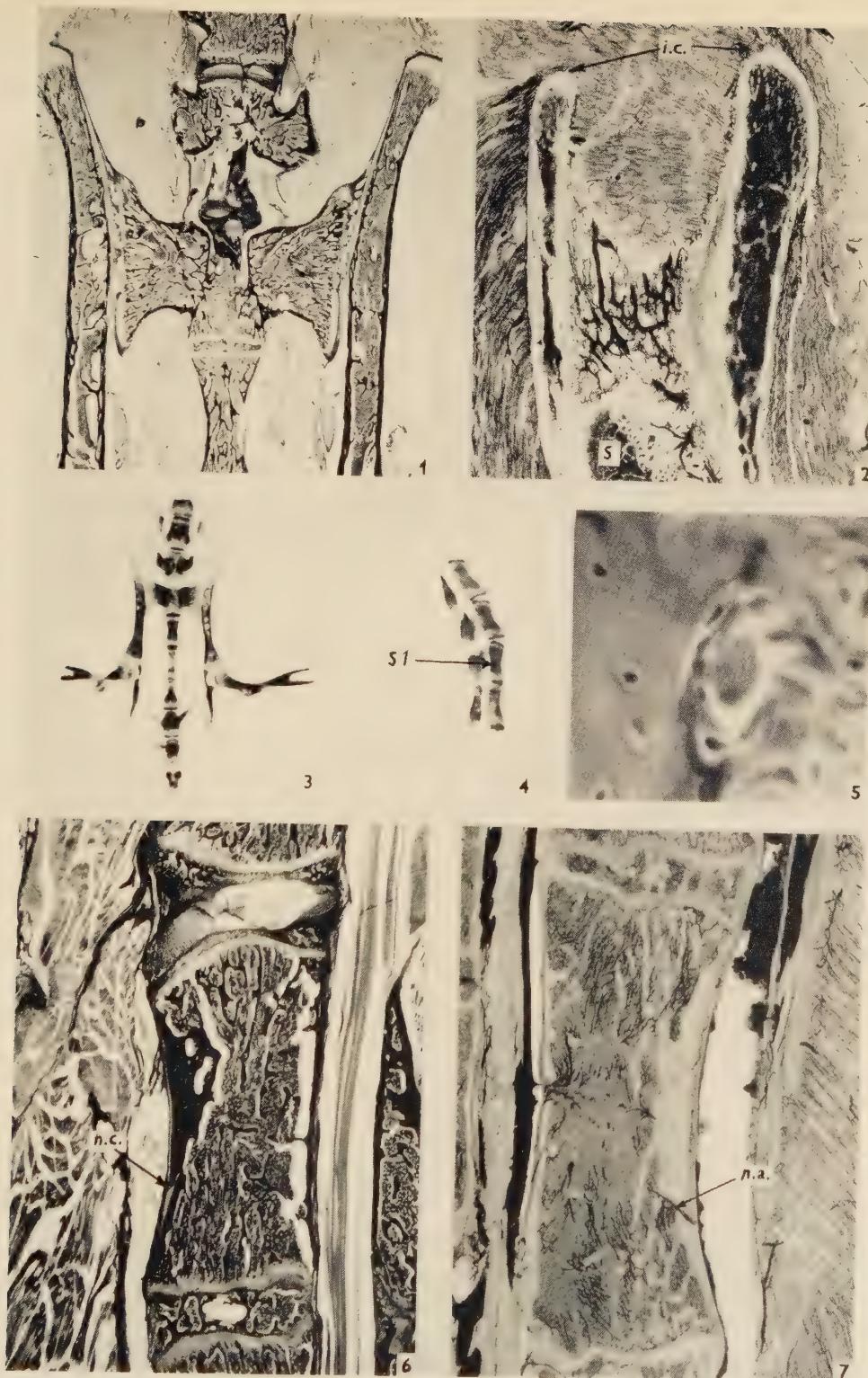
Fig. 17. A transverse section of the ilium just above the level of the sacro-iliac joint in a 10-week-old rat. Weigert's haematoxylin and van Gieson. $\times 27$.

Fig. 18. A sagittal section through the anterior wall of the acetabulum at 90 days. Weigert's haematoxylin and van Gieson. $\times 26$.

Fig. 19. A coronal section through the sacro-iliac joint in a 10-week-old rat. Weigert's haematoxylin and van Gieson. $\times 7.5$.

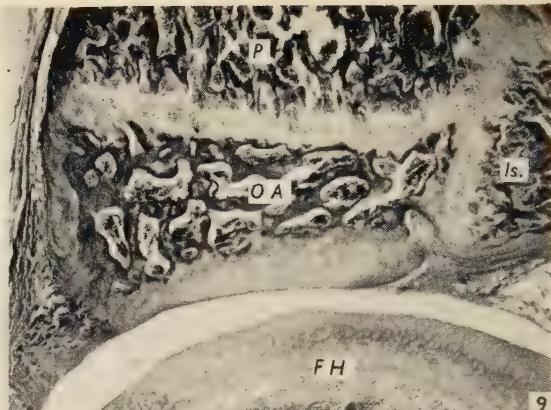
Key to Lettering

A, articular cartilage; *AE*, acetabular epiphysis; *E*, epiphyseal cartilage; *FH*, femoral head; *G*, growth cartilage; *i.c.*, iliac crest; *Il.*, ilium; *Is.*, ischium; *n.a.*, nutrient artery; *n.c.*, nutrient canal; *OA*, os acetabuli; *P*, pubis; *S*, sacrum; *S1*, first sacral body; *S2*, second sacral body; *S3*, third sacral body; *S4*, fourth sacral body; *s.s.l.*, superior sacro-iliac ligament.





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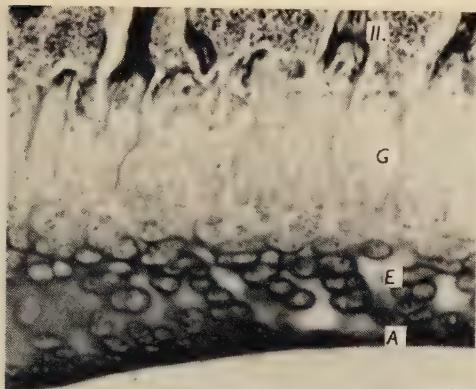
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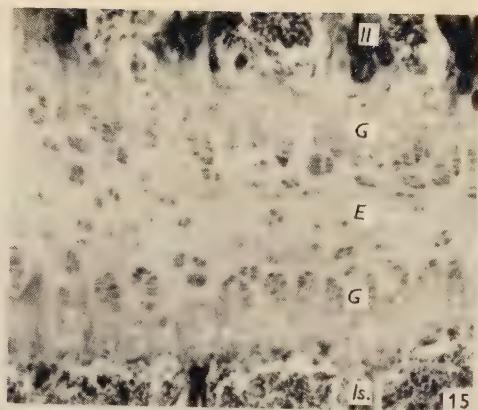
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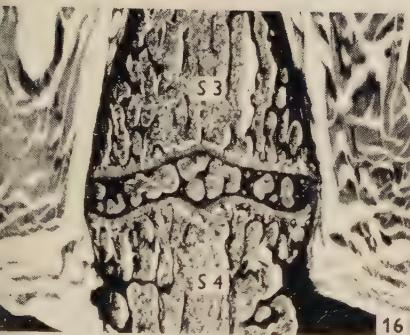
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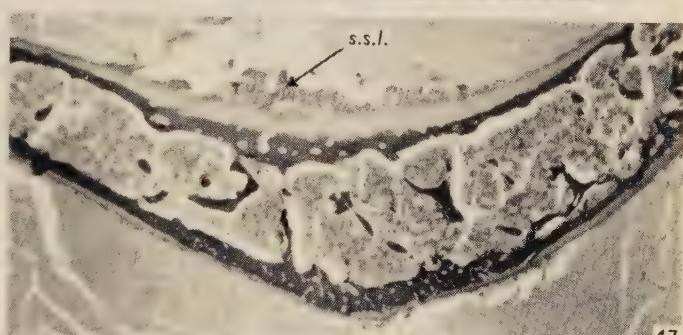
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THE VASCULARIZATION OF LONG BONES IN THE HUMAN FOETUS

BY M. BROOKES

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It is generally held that periosteal arteries play some part in the vascularization of tubular bone cortex, because of a free anastomosis between nutrient and periosteal arterial systems within Haversian canals (Clark, 1952). Estimates of the degree of periosteal nutrition of the compactum usually vary between a half and one-third (Johnson, 1927; Marneffe, 1951). Recently, Brookes & Harrison (1957), using a microradiographic technique, concluded that periosteal arteries play no part in the vascularization of compact bone in the adult rabbit. In this paper the vascular arrangements within tubular bone and the participation of periosteal arteries in compact bone nutrition have been determined in the human foetus. The opportunity was also taken to observe the pattern of vascular cartilage canals in epiphyseal primordia.

MATERIAL AND METHODS

A 50% dilution in normal saline of a 20% barium sulphate suspension (Micropaque, Damancy and Co.) was injected into the thoracic aorta of stillborn foetuses which became available for study within 12 hr. of birth. A mercury manometer was attached to the syringe so that the pressure of injection could be controlled at 70–100 mm. Hg. The injection of the radiopaque mass proceeded slowly generally taking 10–20 min. Adequate filling was judged to have occurred by inspecting the appearances of the intestines, which were filled simultaneously with the vessels of the inferior extremities. The preparation was fixed in 5% formol-saline, following which the limb bones were detached, freed of most of the surrounding soft tissues and placed in 5% nitric acid–5% formol solution for decalcification. Whole bones, femora and tibiae, were radiographed on Kodaline film, some with, others without their periosteal coverings. Transverse sections of the bony shaft, and coronal and sagittal sections of cartilaginous epiphyses were microradiographed on Kodak maximum resolution plates, using a microfocus unit incorporating the Ehrenberg and Spear tube. Sections, which were cut by hand, were about 1 mm. in thickness.

The results of this radiological investigation of foetal skeletal elements are derived from twelve foetuses which had been successfully injected without bursting vessel walls or causing undue extravasation of radiopaque material in those parts subjected to radiographic analysis. Their distribution according to crown rump length is as follows: 16 cm. (1), 17 cm. (1), 18 cm. (1), 19 cm. (1), 20 cm. (2), 22 cm. (3), 28 cm. (1), 29 cm. (1), 35.5 cm. (1).

RESULTS

Vascular patterns in diaphyseal bone

Radiographs of entire specimens in which the periosteum remains *in situ* (Pl. 1, figs. 1, 2) prove that the injection technique is adequate for periosteal filling. The vascular plexus in the membrane surrounding the shaft is seen to be composed of vascular circles and longitudinal anastomotic chains along osseous borders, which together make up an open meshwork closely applied to the surfaces of the diaphysis. When the periosteum has been removed and the bones decalcified, radiography permits the intra-osseous vessels to be clearly discerned (Pl. 1, figs. 3, 4). The principal nutrient artery of the tibia breaks up into a leash of small arteries coursing proximally and distally towards the epiphyseal cartilages. There are normally two principal nutrient arteries in the femoral shaft which unite in the medulla and course towards the juxta-epiphyseal zone, repeatedly subdividing (Pl. 1, fig. 5). The latter is an arteriogram of the distal portion of a 20 cm. foetal femur in which intra-osseous veins have failed to fill with radiopaque medium, leaving the arterial arrangements in the metaphysis unobscured. It shows how the branching of the principal nutrient artery is considerably augmented by the addition of metaphyseal arteries, resulting in the formation of a metaphyseal arterial cone. This is usually visualized radiologically in foetal long bones as a compound arterial and venous structure (Pl. 1, fig. 3), due to the filling with Micropaque of the tributaries of a central venous sinus, which is seen at mid-shaft level in nearly all specimens. This vessel, of wider calibre than the principal nutrient arterial stem in the medulla, is traceable proximo-distally into the metaphyseal vascular plexus. Pl. 1, fig. 6, is from a portion of the juxta-epiphyseal zone of the same femur seen in Pl. 1, fig. 5. It shows that the metaphyseal cone has a brush-border base where the smallest arterial channels abut against the presumptive growth cartilage. Comparison between the arterial pattern of this metaphysis and the more usual metaphyseal appearances in foetal tubular bone, in which a central venous sinus is almost invariably seen following intra-arterial injection of radiopaque media, demonstrates that the metaphyseal blood spaces are largely venous in nature, and contain a wide arterio-venous pathway probably located in the juxta-epiphyseal zone.

Microradiography of transverse sections of foetal diaphysis shows that, in all ages of specimens examined, the vessels seen in compact bone arise in the medulla and pass outwards, centrifugally, towards the surface of the cortex. Pl. 1, figs. 7-9, from foetuses of c.r. length 16, 18 and 22 cm. respectively, demonstrate the medullary vascularization of diaphyseal bone cortex, as revealed in foetal injection preparations made several hours post mortem. The vascular periosteum is *in situ* in Pl. 1, figs. 8 and 9, yet nevertheless a regular periosteal arterialization of the underlying cortex is not to be seen. Because of the adequacy of the injection technique in filling periosteal vessels (Pl. 1, figs. 1, 2) it is difficult to escape the conclusion that, in the human foetus between the ages of 20 weeks and full-term, tubular bone cortex is arterialized from within, from the medulla, and that cortical nutrition does not depend on the periosteal arterial system.

The description of the foetal medullary arterial system and its relationship to cortical vascularization applied to all foetal specimens examined. No developmental

sequence was discerned, presumably because the intra-osseous vascular patterns of foetal tubular bone had already been established at an earlier date.

Vascular patterns in cartilaginous epiphyses

Vascular cartilage canals are detectable in the epiphyses of the youngest specimen in this series (16 cm., 20 weeks), although in a rudimentary and incomplete form. With increasing age their development proceeds, so that, in foetal femora and tibiae of foetuses whose c.r. length is 22 cm. or over, their overall pattern in cartilaginous epiphyses becomes readily discernible. Only in the pattern of cartilage canals of the head of the femur was a modification noted of the canal arrangement which had been established by 22 cm. c.r. length.

The lower end of the femur is vascularized by four groups of canals, entering at the inter-condylar notch, the supra-patellar surface, and the collateral aspects of the condyles. Pl. 2, fig. 1, is a coronal section through this region of a 35·5 cm. foetus illustrating the pattern of vascularization in the femoral condyles, which in A.P. views of the whole femoral lower extremity tends to be a transverse type. Sagittal sections, however, show smaller arteries radiating from the centre of a condyle towards the corresponding articular surface (Pl. 2, fig. 2, c.r. length 22 cm.). The latter figure further shows a vascular cartilage canal joining with the inferior metaphyseal blood cone. Pl. 2, fig. 3, shows the superior extremity of the tibia canalized by vessels penetrating the intercondylar eminence and the whole circumference of the superior tibial cartilaginous primordium. The pattern here is one of convergence towards the centre of the epiphysis. This applies also to the canal pattern of the inferior tibial epiphysis which receives arteries entering from all sides (Pl. 2, fig. 4). A few enter the medial surface of the malleolus and form a separate group.

At the superior extremity of the femur cartilage canals enter the primordium of the head at its anterior rim in earlier bones, although by 22 cm. c.r. length the femoral head is vascularized from nearly all its circumferences (Pl. 2, fig. 5). In the subsequent development of the capital cartilage canals two major groups can be distinguished, a superior and an extensive inferior, the latter taking a semicircular line of origin at the rim of the head which is thereby supplied anteriorly and posteriorly as well as inferiorly by this arterial group (Pl. 2, fig. 6). The ligamentum teres usually contains at least one prominent artery as well as minor channels, but coronal sections cut so as to include the ligament and the foveal area do not reveal any substantial penetration of teretal arterial derivatives into the head proper beyond the immediate cartilaginous area of attachment of the ligament. Pl. 2, fig. 7, from a 28 cm. foetus, shows the greatest arterial penetration of the fovea capitis afforded by the specimens to hand. In all other cases, arterialization of the caput femoris through the fovea was either trifling or absent.

Arterial channels derived from the metaphyseal blood cone occasionally enter into the vascularization of cartilaginous epiphyseal promordia before birth. Examples of such communicating cartilage canals are shown in Pl. 2, figs. 2 and 8, prepared from coronal sections through the upper and lower extremities of the femur. Such canals were not observed in specimens smaller than 22 cm. c.r. length. They tended to be more numerous in sections cut from the older foetuses of the series.

DISCUSSION

From the results of this investigation it may be concluded that the compact bone of the shaft of foetal long bones is supplied by a medullary arterial system. This comprises the derivatives of the principal nutrient artery augmented by metaphyseal arteries, and is in wide communication with small venous channels in the medulla, which are tributary to a longitudinal central venous channel. In cartilaginous epiphyses vascular canals are well developed by 22 cm. c.r. length, and show a pattern of convergence on to the centre of the primordium. With increasing foetal age communicating canals tend to occur more frequently. In the foetal femoral head a superior and extensive inferior group of arteries, entering at the capital circumference, predominate at birth. Penetration of the fovea by teretial arteries occurs in later foetal months, but only to a minor extent.

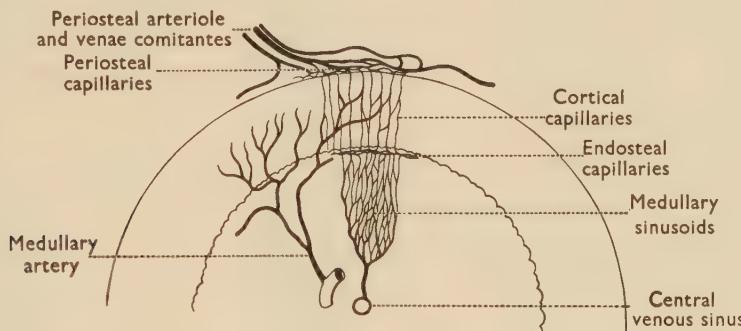
Against the classical concept of a combined periosteal and medullary arterialization of bone cortex (Duhamel, 1743; Hunter, 1772; Ollier, 1867; Langer, 1876; Testut, 1880) may be set the findings of Soulié (1904) who found that the periosteal and medullary systems are quite separate and no anastomosis occurs between them. Moore & Corbett (1914) rejected entirely the notion that bone necrosis follows periosteal stripping. Johnson, in his well-known paper (1927), observed that 'immediate Indian ink injection of the periosteal system was practically ineffectual below the surface of the cortex'. Because of the absence of a periosteal arterialization of the compactum of the adult rabbit (Brookes & Harrison, 1957), and also of the human foetus, it is suggested that the classical doctrine be modified.

In the development of a tubular diaphysis during foetal life two components are recognized. Initially, a perichondral collar of bone is formed followed by a vascular irruption into the cartilaginous shaft. Both perichondral and endochondral bone are laid down around capillaries, which in the earliest phases of bone differentiation are derived from the primitive periosteal and medullary arterial systems. As development proceeds, the medullary capillary network grows *pari passu* with the process of endochondral ossification, until, with the entire removal of the cartilage model, it joins the capillaries in the perichondral collar. The incorporation of periosteal capillaries into the cortical capillary bed during growth in width of the shaft has been described elsewhere (Ham, 1953). The question of the source of arterial blood feeding the cortical capillaries remains.

From the microradiographic evidence it is apparent that in foetal tubular bone fine arterial channels pass into the compactum endosteally; a periosteal arterialization is absent. It is suggested that the whole cortical capillary bed (Text-fig. 1) is fed with arterial blood by the medullary arterial system. Drainage of compact bone is effected either by way of periosteal capillaries, or through medullary sinusoids and the central venous channel. It is emphasized that the vascular systems of bone and periosteum are united, but only at the capillary level. This explains the survival of outlying bone cells seen by Marneffe (1951) in rabbit diaphysis nourished by the periosteum alone, and provides a basis for the development of a collateral circulation, as in Johnson's dog experiments.

The generality of standard English text-books of anatomy and histology state that cartilage is a relatively avascular structure, little or nothing being mentioned

of the presence of vascular cartilage canals. Gray (1954) is exceptional in this respect. It is, however, accepted by workers in this field that cartilage canals have a nutritive and ossific function. Because of their early appearance (Haines, 1934; Hintzsche, 1927; Bidder, 1906) they are not causally related to the ossification process, whose regular onset would appear to be governed by intrinsic mechanisms for each epiphysis.



Text-fig. 1. The blood vascular organization of diaphyseal tubular bone represented diagrammatically in transverse section.

It is possible that the pattern of cartilage canals bears a relationship to that of the larger arterial channels in bony epiphyses (Lexer, Kuliga & Türk, 1904; Rogers & Gladstone, 1950; Trueta & Harrison, 1953). In the case of the caput femoris numerous authors have placed emphasis on the vessels penetrating the bone below the articular rim. In the human foetus this pattern is established by 22 cm., and, in its subsequent development into a superior and inferior group, is closely similar to the arterial conditions in the adult femoral head.

It is by no means settled whether teretal arteries enter the fovea capitis femoris. In the foetus, at any rate, some penetration of arterial channels in the ligamentum teres into the fovea undoubtedly can occur in the later months, although this blood route is only subsidiary in nature.

SUMMARY

1. Human foetal diaphyseal cortex is arterialized from the medulla. A periosteal arterial supply is absent.
2. The foetal metaphyseal blood cone is largely venous in nature.
3. The general pattern of cartilage canals is established by 22 cm. c.r. length.
4. Arterial penetration of the fovea capitis from the ligamentum teres occurs in the later foetal months, but only to a minor extent.

It is a pleasure to acknowledge the encouragement and guidance given me by Prof. R. G. Harrison throughout the course of this research. My thanks are due to Messrs L. G. Cooper, A. Taunton and H. Cowle for their technical assistance and to Mr D. J. Kidd for preparing Text-fig. 1. The research was aided by a grant from the Dora Garrod Thomas Trust.

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EXPLANATION OF PLATES

PLATE 1

The vascularization of osseous portions of the human foetal skeleton as shown by radiography and microradiography following injection of Micropaque into the thoracic aorta and subsequent decalcification.

Fig. 1. Femur. Foetus c.r. length 22 cm. The periosteal membrane has not been removed. Transverse vessels seen at mid-shaft level give an erroneous impression of a periosteal vascularization of the diaphysis. $\times 1\cdot6$.

Fig. 2. Tibia and fibula of same specimen, periosteum *in situ*, showing longitudinal and transverse periosteal vessels. $\times 1\cdot6$.

Fig. 3. Femur. Foetus c.r. length 22 cm. The periosteum has been entirely removed. Upper and lower metaphyseal blood cones are demonstrated. The central venous sinus, superimposed on the principal nutrient arteries, is visible in the middle portion of the diaphysis. $\times 1\cdot6$.





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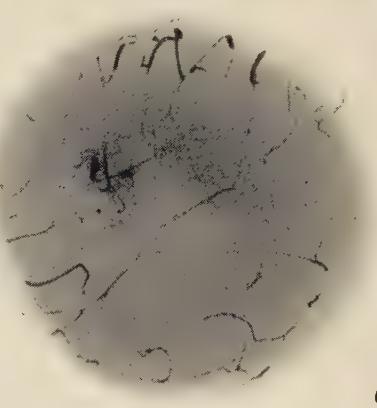
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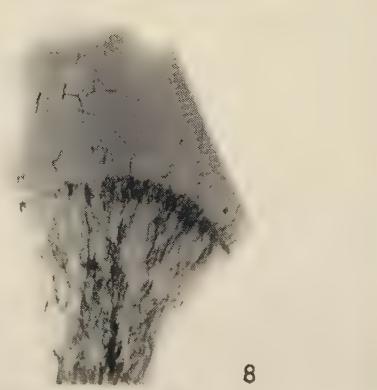
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Fig. 4. Tibia of same specimen as fig. 3, radiographed after periosteal stripping. The principal nutrient artery is finer and denser in the photograph than the closely adjacent central venous sinus, in which the injection mass has formed a broken column. Both vessels are traceable into the metaphyseal blood cones. $\times 1\cdot6$.

Fig. 5. Femur. Foetus c.r. length 20 cm. Two principal nutrient arteries are seen united in the medulla by a fine channel. The metaphyseal blood cone is represented here only by its arterial constituents, branches of the principal nutrient artery and several metaphyseal arteries. $\times 3$.

Fig. 6. Metaphyseal-epiphyseal junction of the femur shown in fig. 5. The terminal arterioles of the metaphyseal blood cone give to the latter a brush-border base abutting against the epiphyseal cartilage. $\times 4\cdot4$.

Fig. 7. Tibia. Foetus c.r. length 16 cm. Periosteum stripped. Transverse section through mid-diaphysis. Medullary arteries alone penetrate into the compactum. Microradiogram, $\times 13\cdot3$.

Fig. 8. Femur. Foetus c.r. length 18 cm. Periosteal arteries clearly demarcate the surface of this transverse section through the mid-diaphysis. Fine medullary arteries pierce the endosteal face of the compactum. No periosteal arterial twigs penetrate into the bone cortex. Microradiogram, $\times 12$.

Fig. 9. Tibia. Foetus c.r. length 22 cm. Transverse section, periosteum and soft tissues present. The cortex is arterialized only from the medulla. Microradiogram, $\times 12$.

PLATE 2

The arrangement of vascular cartilage canals in foetal epiphyses as shown by radiography and microradiography following intra-arterial injection of Micropaque.

Fig. 1. Foetus c.r. length 35·5 cm. Coronal section through inferior epiphysis of femur showing arteries irrigating the cartilage from the collateral condylar surfaces and the intercondylar notch. Microradiogram, $\times 4$.

Fig. 2. Foetus c.r. length 22 cm. Sagittal section through a femoral condyle. Fine arterial channels radiate towards the articular surface. A communicating canal is present. Microradiogram, $\times 4\cdot5$.

Fig. 3. Foetus c.r. length 22 cm. Superior epiphyseal primordium of tibia vascularized from the intercondylar notch and its whole circumference. $\times 4\cdot5$.

Fig. 4. Foetus c.r. length 22 cm. Inferior tibial cartilaginous epiphysis. Vascular cartilage canals enter the malleolus and the sides of the epiphyseal primordium. $\times 4\cdot5$.

Fig. 5. Foetus c.r. length 22 cm. The head of the femur is vascularized from throughout its circumference. The ligamentum teres is *in situ*. $\times 4\cdot5$.

Fig. 6. Foetus c.r. length 35·5 cm. Panoramic view of head of femur which has been removed by sectioning the neck. Superior and inferior groups of arteries predominate in its vascularization. The attachment of the ligamentum teres is still present. $\times 4$.

Fig. 7. Foetus c.r. length 28 cm. Coronal section of head of femur including the fovea, showing a minor penetration of teretal arterial twigs into the cartilage. $\times 5\cdot2$.

Fig. 8. Foetus c.r. length 35·5 cm. Coronal section of upper end of femur. The head has been removed. A few communicating arteries uniting the metaphyseal and epiphyseal systems can be seen. Microradiogram, $\times 2\cdot5$.

THE FINE STRUCTURE OF THE PANETH CELL

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INTRODUCTION

Paneth cells were first described by Schwalbe (1872), later more fully by Paneth (1888), and they have since been found in the intestines of a wide variety of animals.

Their characteristic feature is the large granules, visible in both the fresh and fixed state, in the apical or upper* part of the cell, (Paneth, 1888). The granules are soluble in ether, alcohol, or dilute acids, and are stained well by iron haematoxylin (Klein, 1906). They contain amino acids or protein, as they give a positive ninhydrin reaction (Hintzsche & Anderegg, 1938).

Paneth cells are considered to be exocrine cells, and apart from their situation, their reaction to feeding experiments implies that they are directly concerned with digestion. Klein (1906) found that, after feeding, the Paneth cells of the mouse discharged their granules into the intestinal crypts. Mols (1930) confirmed this, and in addition showed that there was a maximum discharge of granules after a protein meal, a lesser response to a fatty meal, while a carbohydrate meal was ineffective. However, Miram (1912), also working with mice, stated that fat and not protein is the main stimulus to the 'excretion' of Paneth granules. Cordier (1923) records the discharge of Paneth granules following injection of pilocarpine. Van Weel (1937) concluded from enzyme studies that the Paneth cell secretes a peptidase, and therefore aids the digestion of protein.

Mols (1930) undertook the difficult task of trying to find out with the light microscope about the intracellular formation of the large granules, and he concluded that the mitochondria in the upper part of the cell became swollen, divided, and finally converted into the typical granules.

This work deals with the investigation of the ultrastructure of the Paneth cell, at the high resolution now possible with the electron microscope, together with observations on the formation of the secretory granules within the cell.

MATERIALS AND METHODS

Healthy adult mice, 6–9 months old were fasted for 18 hr. and then killed by a blow on the head. Small cubes, 1 mm.³ in size, were removed from the proximal jejunum, 8–10 cm. from the pylorus, and placed in fixative within 2 min. of death.

Fixation and Embedding

The fixative used was an isotonic 1% osmium tetroxide solution buffered to pH 7·2 with veronal acetate. After 4 hr, fixation, the specimen was washed with distilled water, and then dehydrated through graded ethyl alcohols. Following

* For descriptive purposes, in this paper, the part of the Paneth cell resting on the basal membrane is the basal or lower part, and the apical or upper part is next to the crypt.

several changes of *n*-butyl methacrylate it was embedded in a mixture of pure *n*-butyl methacrylate containing 5% methyl methacrylate, and 1% benzoyl peroxide as catalyst. Polymerization was carried out at 45°C., taking about 12 hr.

Sectioning

Preliminary trimming of the specimen block was performed, and thick 10 μ sections cut and examined with the light microscope at $\times 400$ magnification to locate the bases of the crypts. The block was then finally trimmed, and thin sections cut with a thermal-expansion Cook and Perkins microtome. Only silvery white sections, about 200 Å in thickness, were selected. Glass knives were used (Latta & Hartmann, 1950). The sections were mounted on formvar or collodion-covered copper grids for examination.

Electron Microscopy

Survey micrographs were taken on a Philips electron microscope with a 50 Å lens, at magnifications of up to 5000 and enlarged photographically seven times.

High resolution micrographs were taken with a Siemens Elmiskop I electron microscope at direct magnifications of up to 40,000, and enlarged as required.

OBSERVATIONS

Four types of cell can be seen in the lining epithelium of the intestinal crypts of the mouse jejunum—the ‘principal’ or unspecialized cells, Paneth cells, mucous cells, and intermediate cells—which have some of the features of both the mucous and the Paneth cell.

White blood cells, with small dense granules, are occasionally interposed between the bases of epithelial cells. No argentaffin cells were seen, as this study was confined mainly to the bases of the crypts, where they are rarely found (Schofield, 1952). A typical cross-section through the base of the crypt (Pl. 1, fig. 1) shows two or three Paneth cells in a ring of about eight principal epithelial cells. The Paneth cell is readily distinguished by its large secretory granules, well-developed α -cytomembranes, irregular nucleus, and darker cytoplasm.

The typical mucous cell has light, more closely packed granules, while the intermediate cell has granules with a dense central particle and a lighter cortical zone.

THE PANETH CELL

The Cell Membrane

The cell membrane of the Paneth cell, like that of the other epithelial cells, appears as a thin electron dense or osmophilic line, separated from a thin homogeneous basement membrane by a narrow lighter zone. The basement membrane lies between the cell and the sub-epithelial space, which contains collagen fibres and various cells (Pl. 1, fig. 1).

Laterally the cell membrane is separated from that of the neighbouring cell by a narrow interspace (Pl. 3, Fig. 5; Pl. 2, fig. 4). At the level of the nucleus the cell membrane shows one or more narrow invaginations, forming shelf-like folds, while near the free surface, there are one or more terminal bars, characterized by dense cytoplasm and broadening of the interspace (Pl. 2, fig. 4).

The Microvilli

Microvilli project from the free surfaces of the Paneth cells and other epithelial cells of the crypt (Pl. 2, figs. 2, 4; Pl. 5, fig. 11). The cell membrane which is single elsewhere, splits at the free surface of the cell, to form a triple-layered membrane which bounds the microvilli (Pl. 5, fig. 14). The microvilli are about 0.4μ in length and 0.10μ in diameter, are rather irregularly spaced, and are absent or much reduced where there are subjacent secretory granules (Pl. 5, fig. 13). They number approximately $35/\mu^2$, giving about a four-fold increase of the free surface area. In cross-sections, the microvilli contain several circular profiles (Pl. 5, fig. 14), which represent tubular structures running longitudinally, as in longitudinal section only striations are seen. The microvilli on the free surface of the Paneth cell and adjacent crypt cells are similar to those found on a variety of other epithelial cells such as the absorptive columnar cells of the intestinal villi (Zetterqvist, 1956), distal convoluted tubule cells of kidney (Pease, 1955), gall bladder (Yamada, 1955), mucous cells of the small intestine, and epithelial cells of rat trachea (Rhodin & Dahlman, 1956).

The microvilli of a secretory cell such as the Paneth cell are generally shorter, less frequent and less regularly spaced than those of an absorptive cell such as the columnar cell of the intestinal villus (cf. Pl. 5, figs. 11, 12). Those of the latter cell, which form the intestinal striated border, increase the free surface area fourteen-fold (Zetterqvist, 1956), compared with the approximate four-fold increase in the Paneth cell.

The Nucleus

The nucleus is irregular in outline, with invaginations of the nuclear membrane, which are often tubular (Pl. 2, figs. 2, 3).

The nuclear membrane is double, consisting of two dense lines with an intervening lighter zone. The outer membrane is studded with cytoplasmic particles in its outer surface, and resembles a single α -cytomembrane. The inner membrane has numerous nuclear particles associated with its inner surface (Pl. 5, fig. 10). Pores can be seen on cross-section (Pl. 5, fig. 10) and annuli on tangential sections. These annuli are not as prominent as those found in other mammalian cells such as the dorsal root ganglion cell (Dawson Hossack & Wyburn, 1955), or the hepatic parenchymatous cell (Watson, 1955).

Watson (1955) noted connexions between the outer nuclear membrane and the α -cytomembrane in cells such as the exocrine cell of pancreas which have well-developed α -cytomembranes. No connexions of this kind were found in the Paneth cell, although they were occasionally seen in the principal cells where there are fewer α -cytomembranes.

The nucleolus is a dense sponge-like reticulum.

The Cytoplasm

The main distinguishing features of the Paneth cell are the numerous α -cytomembranes, Golgi complex, large secretory granules, and clusters of dense small granules. The last-mentioned are peculiar to the Paneth cell.

Other inclusions are cytoplasmic particles, mitochondria, vacuole-containing bodies, and cytoplasmic vacuoles.

The α -cytomembranes

A system of paired membranes, arranged concentrically on the nucleus, fill most of the basal two-thirds of the cell (Pl. 1, fig. 1). These are the α -cytomembranes of Sjöstrand (1956), or the granular profiles of the endoplasmic reticulum (Palade 1956). On section, each membrane pair below the nucleus lies parallel to the basement membrane, and extends upwards around the nucleus; the inner pairs encircling it, while the outer ones continue upwards into the upper third of the cell and end among the secretory granules (Pl. 1, fig. 1; Pl. 2, figs. 2, 3).

Each membrane has a smooth surface and a surface studded with small opaque particles. The membranes of each pair are continuous with one another at their ends and so enclose a flattened space lined with the smooth surfaces, while the rough surfaces face the cytoplasmic ground substance (Pl. 2, fig. 4; Pl. 5, fig. 10). The membrane pairs among the secretory granules are disposed at random, are shorter, and appear as circular or elongated profiles (Pl. 2, fig. 2).

The Golgi Complex

Dalton & Felix (1954) and Sjöstrand & Hanzon (1954) have described the ultra-structure of the Golgi complex, which has subsequently been identified in a wide variety of cells.

The Golgi complex in the Paneth cell is extensive, and occupies a discoidal space above the nucleus, the plane of the disc being transverse (Pl. 1, fig. 1). It consists of several smooth membrane pairs, each pair being closed at its ends to form a flattened sac. There is no communication between adjacent membrane pairs. The flattened sacs may be expanded at their margins to form the Golgi vacuoles. Other vacuoles on section have their own limiting membrane and appear to be independent of the membrane pairs. Small Golgi vesicles surround the membranes and vacuoles (Pl. 4, figs. 7, 8, 9). These vesicles or granules are from 450–500 Å in diameter. Smaller vesicles about 100 Å diameter are also found.

The Golgi complex of the adjacent principal cells has similar structural elements, but is more compact, smaller, and has relatively more vacuoles and fewer vesicles (Pl. 1, fig. 1; Pl. 3, fig. 6) than that of the Paneth cell.

The Secretory or Paneth Granules

The secretory granules are the most prominent feature of the Paneth cell, and fill the upper third of the cell (Pl. 1, fig. 1; Pl. 2, figs. 2, 4). They are dense spherical bodies of 0·75–1·5 μ in diameter, each lying in a vacuole of 1–2 μ in diameter (Pl. 2, fig. 4). Some of the vacuoles, especially the smaller ones, have a single enclosing membrane (Pl. 4, fig. 7).

The vacuole is not a constant feature, however, as there are occasional cells where the space surrounding the granule is filled with a moderately osmophilic material (Pl. 2, fig. 2). The appearance of the granule with its surrounding vacuole was the same after fixation in Palade's hypotonic fixative or Dalton's osmium-chromate solution, and washing with Tyrode's solution instead of distilled water.

Formation of Secretory Granules

Survey micrographs show that in addition to the large secretory granules in the upper third of the cell, other secretory granules and related vacuoles lie within the Golgi region. These, in contrast to the large granules, are smaller, often irregular in outline and lie eccentrically within their vacuoles, which have an enclosing membrane (Pl. 2, figs. 3, 4). Three types can be seen:

(1) In close relation to the Golgi membranes are vacuoles enclosed by a single membrane, which sometimes is partly deficient. These vacuoles contain a cluster of small vesicles at one part of their circumference. The contained vesicles are similar in shape and size to the Golgi vesicles (Pl. 4, fig. 7).

(2) The second type of vacuole is similar to the first in having an enclosing membrane which is sometimes partly deficient, but contains, in addition to the cluster of vesicles, some of the fine granular substance typical of the large secretory granule. An example is seen in Pl. 2, fig. 4, where the deficiency in the enclosing membrane at the site of the cluster of vesicles allows them to communicate freely with the adjacent Golgi vesicles.

(3) The small secretory granules within the Golgi region have, embedded within their finely granular substance, vesicles similar to the Golgi vesicles (Pl. 4, figs. 8, 9). As in the case of the previous two types of vacuole, the enclosing membrane may be deficient, allowing continuity between the contents of the granule and the adjacent Golgi ground substance and vesicles (Pl. 4, fig. 8).

Thus it appears that the large secretory granule develops from a vacuole arising within the Golgi complex. First the vacuole incorporates Golgi vesicles through a deficiency in its enclosing membrane (Type 1). Secondly it comes to contain finely granular substance in addition to the cluster of vesicles (Type 2). Thirdly, by further accumulation of granular substance it becomes a small secretory granule in which vesicles can still be seen embedded in the mass of granular substance (Type 3).

As the small secretory granule increases in size it moves upwards towards the free surface of the cell, comes to lie symmetrically within its vacuole, and prior to its extrusion into the crypt loses its enclosing membrane.

Clusters of Dense Particles

Apart from the typical granule, the Paneth cells have characteristic clusters of very dense small granules arranged irregularly, usually within a vacuole. Each cluster is about the size of a large secretory granule, and the average section shows two or three clusters in the upper part of each Paneth cell (Pl. 2, figs. 3, 4). There is no evidence that they are extruded into the crypt, or transformed directly into secretory granules.

Cytoplasmic Particles

Small opaque particles about 175 Å in diameter are found free in the cytoplasm of the Paneth cell (Pl. 5, fig. 10). They are similar to the particles attached to the α -cytomembranes, and most of the apparently free particles are obliquely-sectioned α -cytomembranes. In contrast, the cytoplasm of the neighbouring principal cells has abundant small groups of free particles (Pl. 3, fig. 5).

Palade (1955) considers that both the free and attached particles contain large amounts of RNA, and so constitute the basophilic component of the cytoplasm.

Mitochondria

Rod-shaped mitochondria are scattered throughout the basal two-thirds of the cell (Pl. 1, fig. 1; Pl. 2, fig. 3). Below the nucleus, they lie transversely, but elsewhere those close to the cell membrane are parallel to the long axis of the cell, while the others are dispersed at random. Their ultra-structure is typical of mitochondria in other cells (Pl. 3, fig. 5). They are bounded by an outer double membrane and have numerous inner double membranes or cristae, and their ground substance is denser than that of the cytoplasm (Pl. 2, fig. 4). Dense granules lie in the mitochondria of the principal cells, but are rare in the Paneth cell (Pl. 2, fig. 3; Pl. 3, fig. 5). Weiss (1955) concluded that similar granules in duodenal epithelial cells were involved in cation transport.

Vacuole-containing Bodies

These consist of a vacuole about $0\cdot3$ – $0\cdot5\mu$, bounded by a single membrane, containing numerous smaller vacuoles 300–800 Å in diameter. In the majority of sections there are one or more of them in the upper half of the cell, often among the secretory granules (Pl. 2, fig. 4).

Similar structures have been described in the neurone by Palay & Palade (1955), in the gall bladder epithelium by Yamada, (1955), and in the tracheal epithelial cells by Rhodin & Dahlmann (1956), who introduced the term vacuole-containing body.

In the present study they were also found in the neighbouring principal cells. Zetterqvist (1956) described similar structures in the columnar absorbing cell of the intestinal villus, and noted that the smaller ones about $0\cdot3\mu$ were close to the Golgi complex.

In the Paneth cell no small vacuole-containing bodies were found in the Golgi complex.

Cytoplasmic Vacuoles

Vacuoles about $0\cdot1$ – $0\cdot2\mu$ in size with an enclosing membrane are common in the upper part of the cell close to the free surface (Pl. 2, fig. 4; Pl. 5, fig. 11).

DISCUSSION

The secretory granules and well-developed α -cytomembranes of the Paneth cell indicate its exocrine nature. The detailed arrangement of the α -cytomembranes, for example, is strikingly similar to that in the exocrine cells of pancreas, Sjöstrand & Hanzon (1954). The Golgi complex of the Paneth cell is more extensive than that of the non-secretory principal epithelial cells, and has a relatively higher proportion of Golgi vesicles, which may well reflect the part these vesicles play in the formation of the secretory substance.

The vacuoles surrounding the secretory granules are not present in other exocrine cells, such as mucous cells, or those of the pancreas. This vacuolation is unaltered

by changes in the tonicity of the fixative or washing fluid, or by a 10 min. delay in fixation. Mention has been made of the occasional cell in which there is no vacuole, but each granule is surrounded by a zone of moderately osmophilic material (Pl. 2, fig. 2). Probably this resembles more closely the *in vivo* condition, and the vacuole results from a loss of this material during processing.

In this connexion it is of interest that a somewhat similar appearance is seen in the electron microscopy of the avian red blood cell, where after osmium tetroxide fixation the nucleus is surrounded by a vacuole or 'halo'. This halo persisted in spite of varying the tonicity, pH, duration, or rapidity of fixation. Centrifugal force was also excluded as a causative factor. Now and then, however—but not in relation to any particular method of treatment—a cell was found in which the 'halo' was minimal, and it is considered that its presence depends on changes in the nucleo-cytoplasmic volume (Hally, 1956, unpublished observations).

On the basis of the appearance of the secretory granules within the Golgi complex, it is suggested that they are developed in the following way. A 'secretory' vacuole arises within the Golgi complex, and incorporates Golgi vesicles through a gap in its enclosing membrane. The vacuole now becomes a small secretory granule, in which vesicles can still be seen embedded in the finely granular substance, which itself may be derived from breakdown of the vesicles. As the secretory granule increases in size, it moves towards the apex of the cell, becomes surrounded by a vacuole, and usually loses its enclosing membrane.

If this interpretation is correct, then the Golgi complex is the site of formation of the Paneth granule, and the Golgi vesicles form the raw material of the secretory substance.

Membranous 'ghosts' of granules lie within the crypt (Fig. 13), so possibly the discharging granule evaginates the cell membrane, which comes to enclose it and is 'pinched off' as the granule is finally extruded into the crypt.

The present study does not support the view of Mols (1930) that the secretory granules arise directly from mitochondria.

Weiss (1953) studied the exocrine cells of mouse pancreas, and concluded that the secretory zymogen granules were formed from the 'ergastoplasmic sacs' (α -cytomembranes). However, at that time the Golgi complex had not been conclusively identified electron-microscopically, and possibly the 'ergastoplasmic sacs' included part of the Golgi complex.

Sjöstrand & Hanzon (1954), also studying mouse pancreas, and at a higher resolution, found an intimate relationship between the zymogen granules and the Golgi 'granules' or vesicles. They stressed, however, that the time relationships were unknown, and the same criticism applies to the Paneth cell.

Hagenau & Bernhard (1956) in an interesting survey of the Golgi complex, drew similar conclusions about the cells forming specific granules in the anterior pituitary.

Burgos & Fawcett (1955) have shown that the acrosome of the spermatid arises from a vacuole within the Golgi complex.

Thus accumulating knowledge of the fine structure of secretory cells indicates that the Golgi complex, and especially the Golgi vesicles, contribute to the formation of the secretory products of the cell.

SUMMARY

After fixation with osmium tetroxide, the Paneth cells at the base of the crypts in mouse jejunum were examined with the electron microscope.

The principal epithelial, mucous and intermediate cells are briefly mentioned.

The ultra-structure of the Paneth cell is typical of an exocrine sero-zymogenic cell. It is characterized by large secretory granules with surrounding vacuoles, numerous α -cytomembranes, and clusters of small dense particles.

It is suggested that the secretory granule of the Paneth cell arises from a vacuole within the Golgi complex, which incorporates Golgi vesicles and becomes a small secretory granule. As it increases in size the maturing granule moves towards the apex of the cell, becomes surrounded by a vacuole, and is finally extruded into the crypt.

Thus the Golgi vesicles, a component of the Golgi complex, contribute directly to the final secretory product of the cell.

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EXPLANATION OF PLATES

PLATE 1

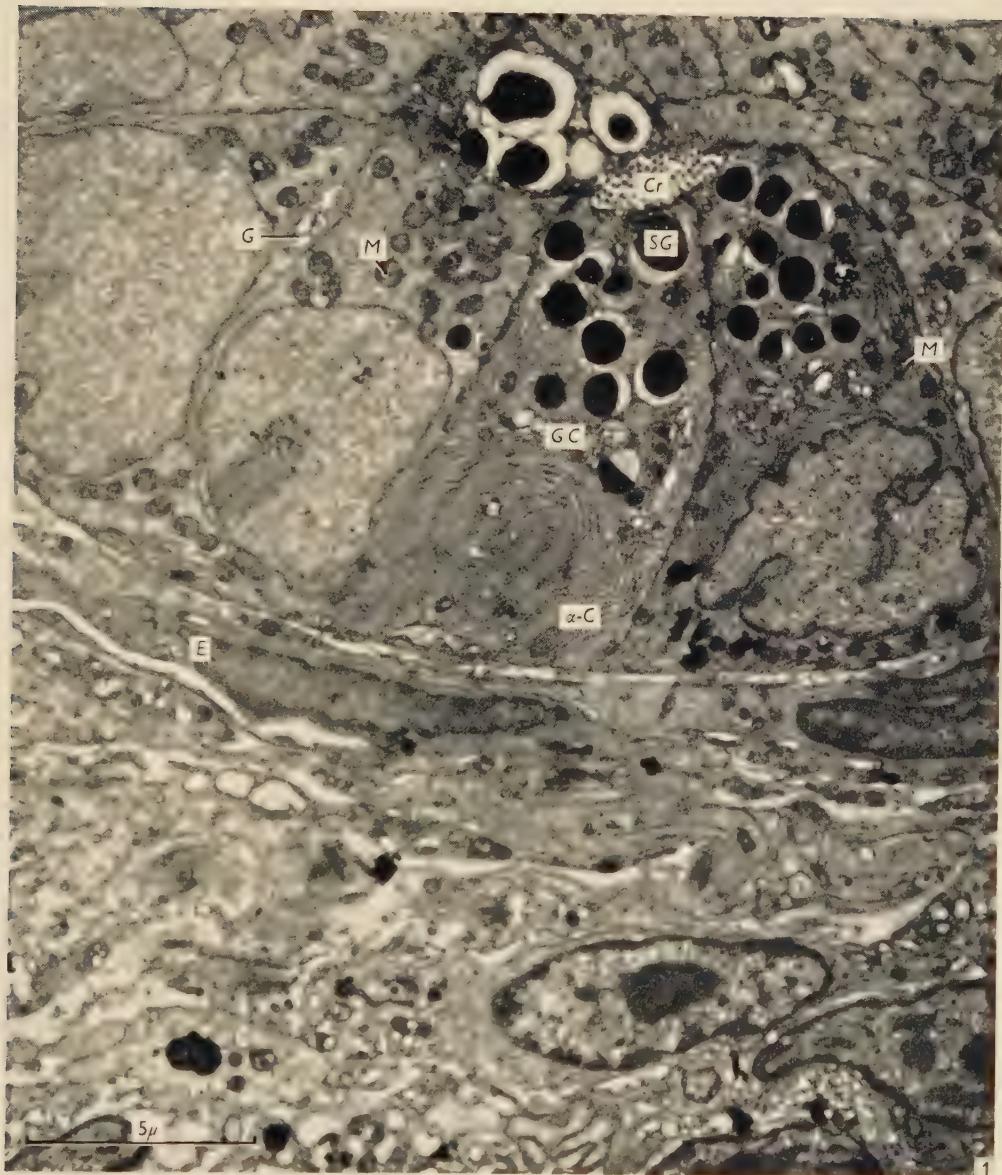
Fig. 1. Survey picture through base of a crypt (*Cr*), with two Paneth cells and the upper part of a third, interposed in a ring of about eight principal epithelial cells. The Paneth cells are distinguished by large secretory granules (*SG*), within surrounding vacuoles, numerous α -cytomembranes (α -*C*), irregular nucleus, and darker cytoplasm. The Golgi complex (*GC*) lies above the nucleus. Mitochondria (*M*) are scattered throughout the epithelial cells of the crypt. Note compact Golgi complex (*G*) of principal cell. The sub-epithelial space, with cells and collagen fibres, fills the lower half of the picture. Note the capillary bounded by an endothelial cell (*E*). $\times 6000$.

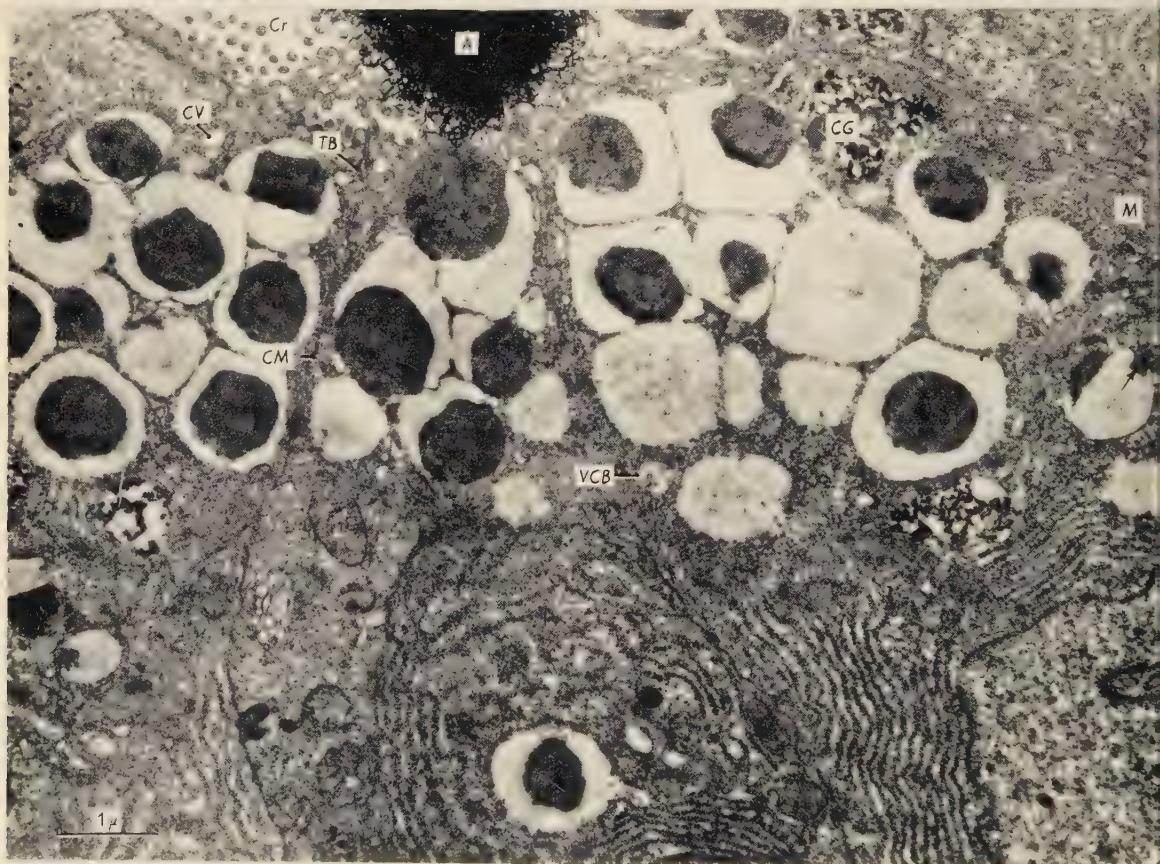
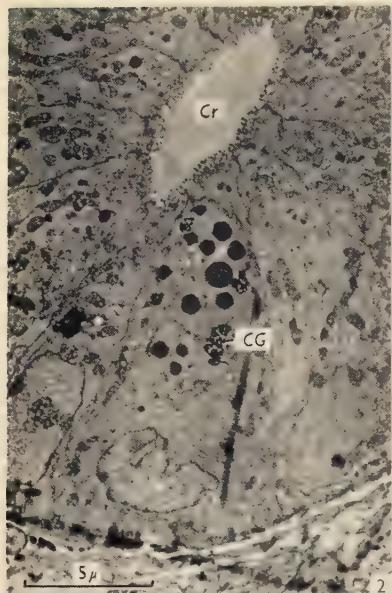
PLATE 2

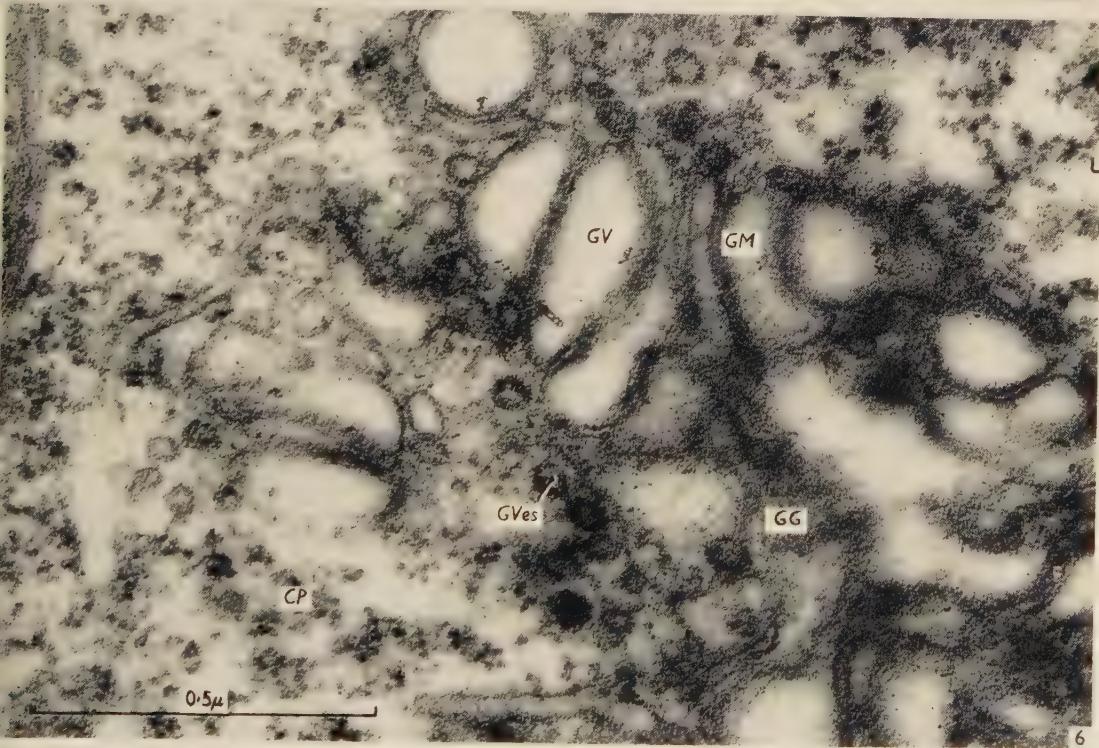
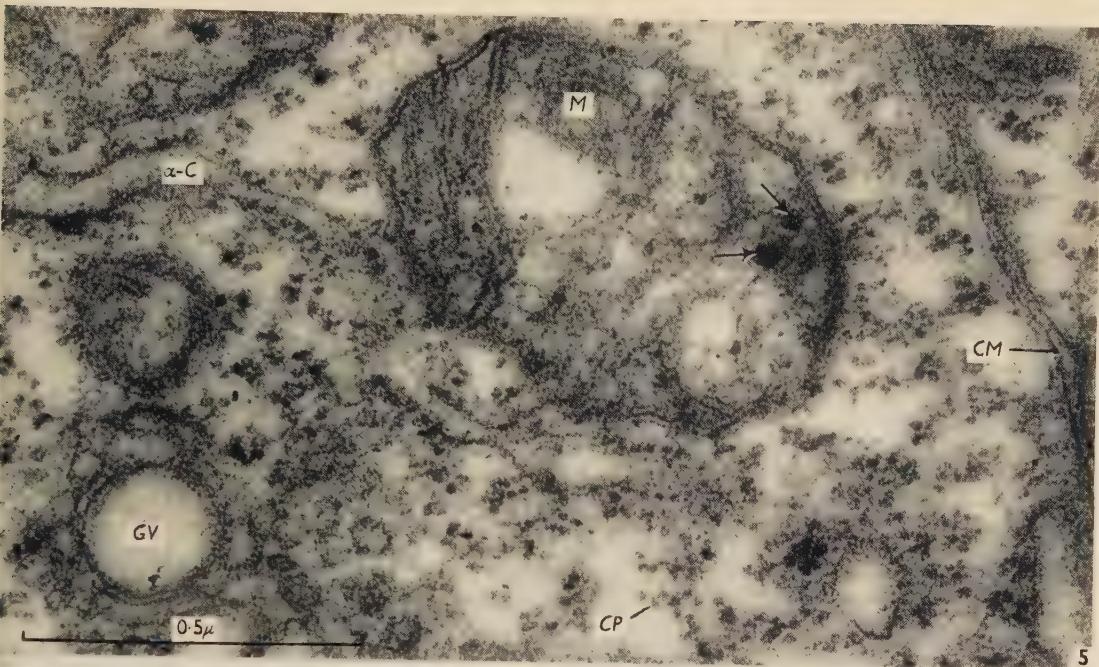
Fig. 2. Survey picture through base of crypt (*Cr*), with a Paneth cell and adjacent principal cells. The Paneth cell contains secretory granules and clusters of small dense granules (*CG*). The α -cytomembranes are arranged concentrically around the nucleus, and at random among the secretory granules. $\times 3300$.

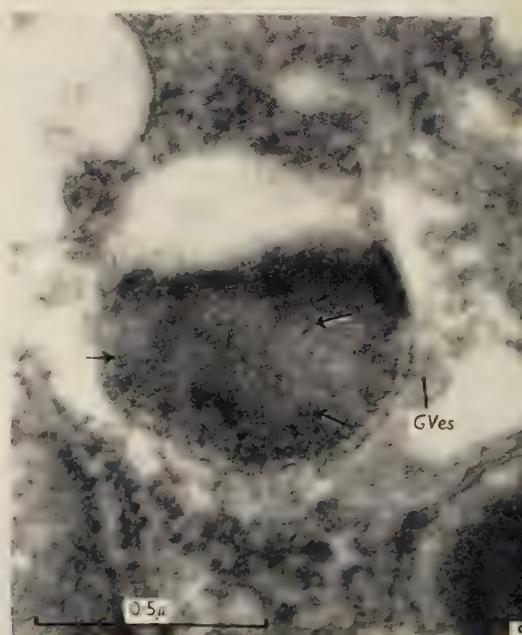
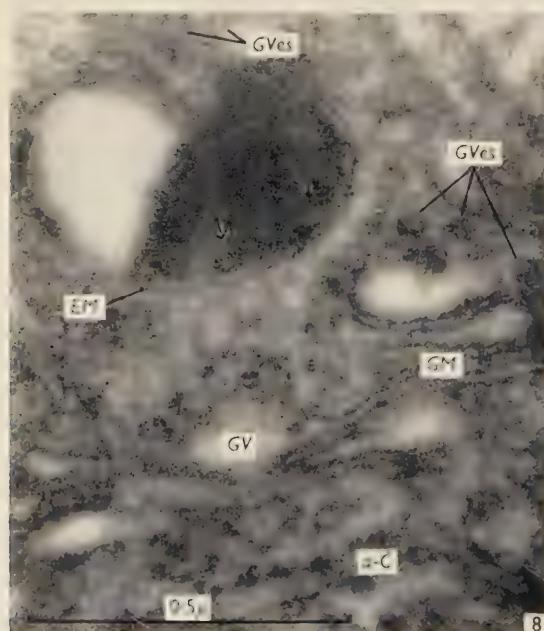
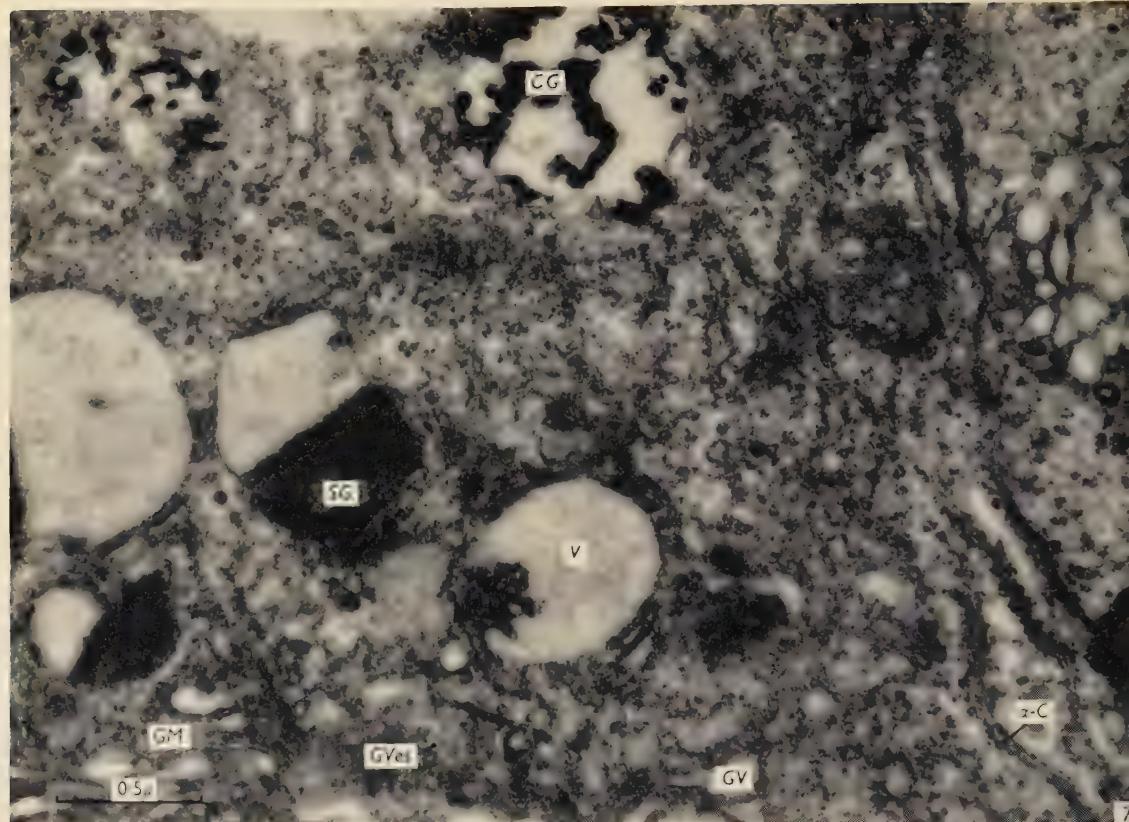
Fig. 3. Survey micrograph of base of crypt below the lumen. Most of the cells are obliquely-sectioned Paneth cells. Certain cells with no secretory granules are readily identified as Paneth cells by their numerous α -cytomembranes (α -*C*). Small secretory granules (arrows) lie within the Golgi complex (*GC*), which is above the nucleus. The mitochondria (*M*) of the principal cells contain dense granules, in contrast to those of the the Paneth cell. $\times 4000$.

Fig. 4. Micrograph of the upper part of several Paneth cells converging on a crypt (*Cr*), which contains transversely cut microvilli. Within the Paneth cell are large secretory granules (*SG*), clusters of small dense granules (*CG*), cytoplasmic vacuoles (*CV*), and a vacuole-containing body (*VCB*). The cell membrane (*CM*) is shown, with a terminal bar (*TB*) close to the free surface. Note artefact (*A*).









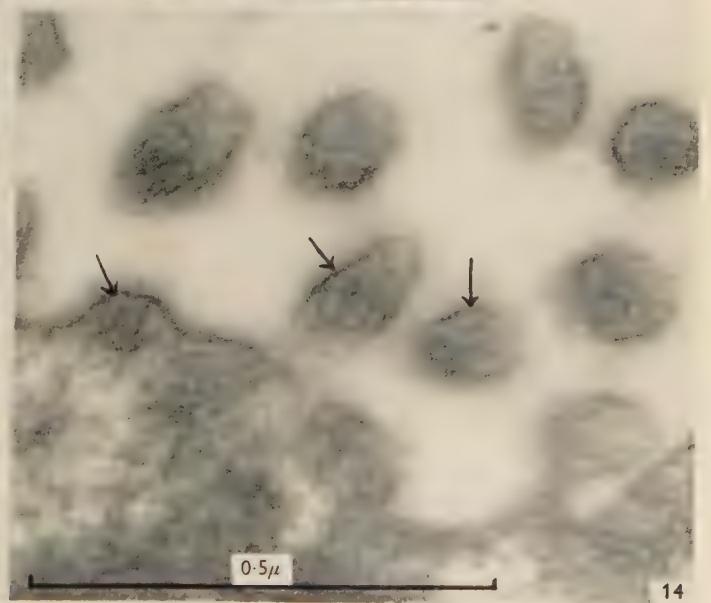
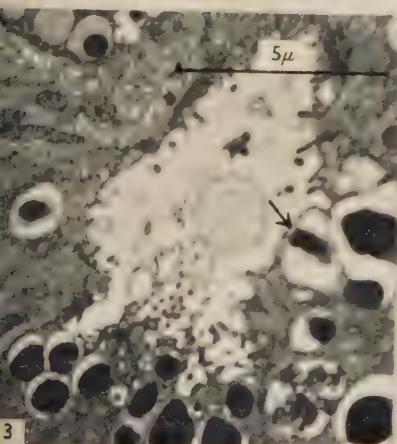
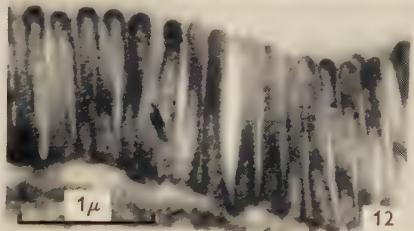
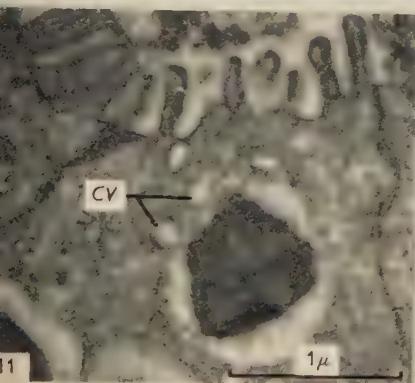
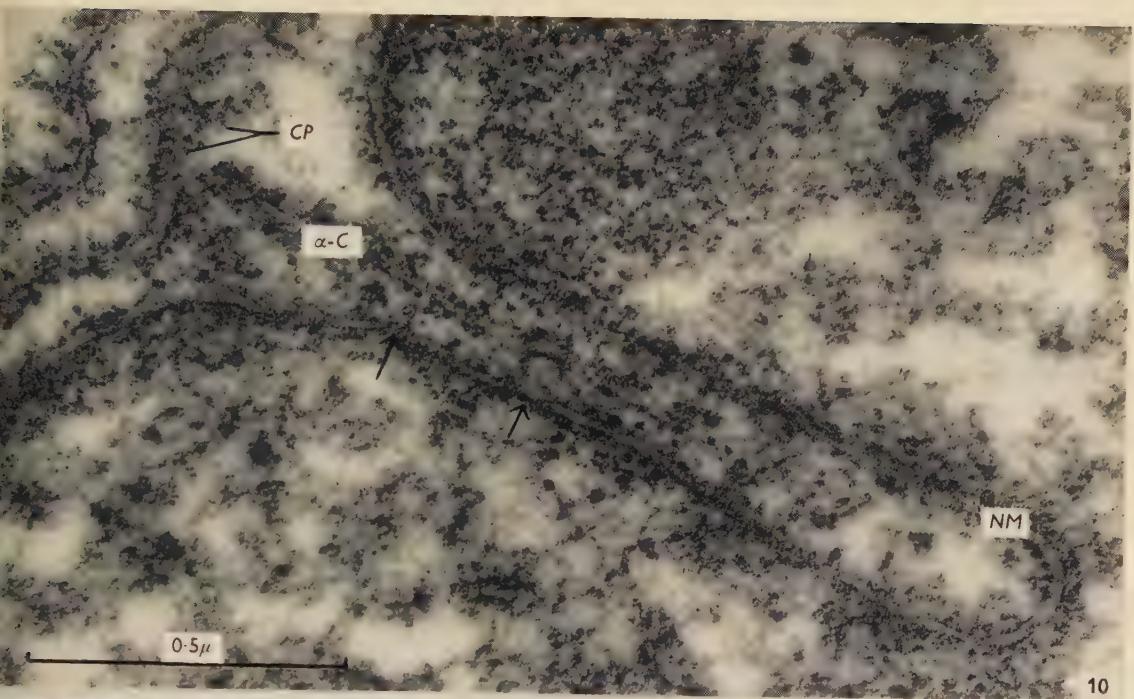


PLATE 3

Fig. 5. Mitochondrion in cytoplasm of a principal epithelial cell. The mitochondrion (*M*) is bounded by a double outer membrane and contains internal double membranes or cristae. Within its ground substance are two dense granules (arrows). Small groups of cytoplasmic particles (*CP*) lie free in the cytoplasm. Similar particles are attached to the surface of α -cytomembranes (α -*C*). A cell membrane (*CM*) is accompanied by that of the adjacent cell. A Golgi vacuole (*GV*) lies at the lower left corner. $\times 100,000$.

Fig. 6. Golgi complex of principal epithelial cell. The smooth Golgi double membranes (*GM*), Golgi vacuoles, (*GV*), and small vesicles (*GVis*), are lying in the Golgi ground substance (*GG*). The latter is free of cytoplasmic particles (*CP*) which are present only in the adjacent cytoplasm. $\times 100,000$.

PLATE 4

Developing Paneth granules in Golgi zone

Fig. 7. Supranuclear region of Paneth cell. The Golgi complex with Golgi vacuoles (*GV*), vesicles (*GVis*), and smooth double membranes (*GM*), in a ground substance which is free of α -cytomembranes and cytoplasmic particles. Small secretory granules (*SG*) lie within the complex.

Note 'secretory' vacuole (*V*), containing a cluster of vesicles similar to the Golgi vesicles (*GVis*), in close relation to Golgi membranes. $\times 37,000$.

Fig. 8. Enlarged view of developing secretory granule from fig. 7 showing granule in close relation to the Golgi vesicles (*GVis*), smooth membrane pairs (*GM*) and vacuoles (*GV*) of the Golgi complex. The granule is irregular in outline, and the enclosing membrane (*EM*) is deficient above, where the osmophilic granular material communicates freely with the Golgi ground substances and vesicles. Embedded in the granule are vesicles similar to the Golgi vesicles (*GVis*).

Fig. 9. Developing granule within Golgi zone. Another secretory granule within the Golgi complex, containing vesicles (arrows), similar to closely related Golgi vesicles (*GVis*).

PLATE 5

Fig. 10. Portion of nucleus of Paneth cell. The nucleus lies to the right, and the cytoplasm at the upper left hand corner. The nuclear membrane (*NM*) is double, and is interrupted by pores (arrows). It is invaginated into the nucleus by a long process of cytoplasm, which contains an α -cytomembrane pair (α -*C*). The α -cytomembranes have smooth inner surfaces and outer surfaces studded with cytoplasmic particles (*CP*). Note also free cytoplasmic particles. $\times 80,000$.

Fig. 11. Upper part of Paneth cell showing microvilli projecting from free surface into crypt. Cytoplasmic vacuoles (*CV*) are close to the free surface of the cell. $\times 22,000$.

Fig. 12. Picture of the microvilli forming the brush border of the columnar cells of the intestinal villus, which are more closely packed, regularly spaced, and longer than those of the Paneth cell (cf. fig. 11). $\times 17,000$.

Fig. 13. A crypt bounded by several Paneth cells. A membranous 'ghost' lies within the crypt. Notice the absence of microvilli on the free surface over a subjacent secretory granule (arrow). $\times 5,400$.

Fig. 14. High resolution picture of obliquely-sectioned microvilli of Paneth cell. A triple-layered membrane bounds the free cell surface and microvilli (arrows). $\times 115,000$.

OBSERVATIONS ON THE HISTOCHEMISTRY AND FINE STRUCTURE OF THE NOTOCHORD IN RABBIT EMBRYOS

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INTRODUCTION

Although the main facts of the development and histology of the notochord are well known, there appears to be a sparseness of data concerning its histochemistry and fine structure. The notochord has been studied in a variety of animal species but the workers concerned have confined themselves in general to the use of standard staining procedures.

The axial rod of cells which makes up the notochord during the period when the somites are laid down becomes modified as the primordia of the vertebral centra and intervertebral discs are established. Carlier (1890) described the formation of curious V-shaped bends of the rod, the point of the V directed posteriorly, at the sites of the future intervertebral discs in early sheep embryos. He quoted Balfour (1876) and Köllicker (1882) as having noted the same phenomenon. In a sheep foetus of 17·5 mm. c.r. length, Carlier further observed the absence of notochordal cells in the developing centra, but the presence here of a jelly-like matrix which he thought was produced by the perichordal cells. Similar findings were made by Minot (1906) and Williams (1908). The latter, in detailing the histology of the notochord, described it (chiefly in the pig embryo) as first composed of epithelial cells, then turning into a syncytial mucoid connective tissue, and finally becoming 'cellular' again and looking rather like cartilage. He also observed, in one stage of a rabbit embryo, vacuolation of the notochord cells; the notochord sheath is, according to Williams, in the early stages striated concentrically but later develops an inner zone composed of a mucin-like material, staining with mucicarmine, derived from the vacuoles of the notochord cells. Dawes (1930) traced the development of the notochord in the mouse and confirmed the observations of Williams (1908) that it is in turn 'cellular', 'syncytial' and finally 'cellular'. Sensenig (1943) described, in the deer mouse, migration of sclerotomic cells through the notochord sheath and concluded that these cells thus added to the bulk of the nuclei pulposi. The same author (1949), working with human material, found that the development of the sclerotomic perichordal tube coincided with the appearance of a homogenous 'eosinophil elastica externa' and suggested a sclerotomic rather than notochordal origin for this sheath. Peacock (1951, 1952) described the perinotochordal tissue in human embryos as specialized embryonic cartilage, and observed that the notochord cells underwent mucoid degeneration to form the nuclei pulposi. He also found the notochord sheath to stain lightly with basic dyes and to show longitudinal striations. Finally, Duncan (1957), in a study of the early chick embryo with the electron microscope, unfortunately only available as yet in the form of an abstract,

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states that the notochord is surrounded by a halo of ultrafine fibrils. They are 'limited to the vicinity of the notochord' and appear to be 'produced at the surface of the notochord rather than within this structure'.

In view of the relative paucity of information on the actual cytology of the notochord and the structure of its sheath, it was decided to investigate it both histo-chemically and by means of the electron microscope. The present observations concern only rabbit embryos and are confined to the stages in which the notochord is fully established and undergoing changes associated with the production of the definitive nuclei pulposi.

MATERIAL AND METHODS

Embryos were obtained from does of 13, 15 and 17 days after observed matings and the stages of development of the embryos checked with the table of Minot & Taylor (1905). For light microscopy, embryos were fixed whole in alcoholic Bouin, cold absolute ethyl alcohol or cold absolute acetone. They were dehydrated through graded alcohols and embedded in paraffin wax in the usual manner. Sections were cut at 7μ , at least one embryo of each stage being cut transversely and one sagittally. The following staining procedures were used: haematoxylin and eosin, Masson's trichrome, Azan, iron haematoxylin-van Gieson, Best's carmine, mucicarmine, alcian blue, Weigert's elastin, Wilder-Gomori silver impregnation for reticulin, the P.A.S. reaction and both the calcium cobalt and azo-dye techniques for alkaline phosphatase. With the material for electron microscopy, the problem of the preparation of specimen blocks prior to fixation arose. It is possible to dissect out the notochord or, at least, the developing vertebral column of embryos at the stages studied but, by doing so, normal relationships and the structure of the notochord may be altered. It was decided to prepare specimen blocks by removing the head and ventral and lateral portions of the thorax and abdomen of each embryo and then cut transverse sections of the remaining dorsal regions about 0.5–1.0 mm. thick. These blocks of tissue, measuring 0.5–1.5 mm.³, were fixed by immersion in 1% osmium tetroxide, which previous experience had shown to penetrate into embryonic tissue more rapidly than into adult tissue. The osmium tetroxide was buffered to pH 7.2 either with acetate veronal (Palade, 1952) or bichromate (Dalton, 1955) and fixation performed at room temperature for periods of either 45 or 60 min. Washing, dehydration, infiltration with and embedding in 5% methyl in *n*-butyl methacrylate were routine, polymerization being effected at 60° C for 24 hr., at which temperature polymerization damage is minimal (Borysko, 1956). Ultrathin sections were cut on a Sims-Leeson heat-advance ultramicrotome (1957), using glass knives, collected on carbon-coated grids and examined in a Metropolitan-Vickers E.M. 3 electron microscope at 75 kV.

RESULTS

Thirteen-day stage

The embryos studied varied from 8.5 to 11.1 mm. c.r. length before fixation. In cross-section, the general shape of the notochord is circular and in a longitudinal section there is no evidence of segmentation, i.e. there is no intervertebral enlargement and no intravertebral constriction. In a 7μ transverse section (Pl. 1, fig. 1) there are approximately 12–14 markedly chromatic nuclei which are large, circular,

or nearly so. In a longitudinal section (Pl. 1, fig. 4: an electron micrograph), they appear oval in a transverse direction indicating a biconvex disc shape. The cells are arranged in a pattern which is strikingly epithelial in character. Cell boundaries are difficult to define on light microscopy (Pl. 1, fig. 1) but are clearly seen in the low-power electron micrograph of Pl. 1, fig. 4. Small vacuoles are present here and there in the cytoplasm. The notochord sheath is well marked, appears homogeneous and undulates over the contours of the notochord cells. It is eosinophil and stains red with van Gieson, intensely green with Masson's trichrome, blue with azan stain and with alcian blue, and red with mucicarmine. It shows a positive P.A.S. reaction. With Weigert's elastin, some of the more peripheral parts of the sheath stain in places pale purple. Neither in the sheath nor in the cells is there any alkaline phosphatase activity. A condensation of mesenchymal cells around the sheath of the notochord forms the perichordal tube. These cells are arranged concentrically and the nuclei are oval in shape. The notochord is avascular in this and all other stages examined.

In high-power electron micrographs, the nuclear membrane is double and the notochord cells show clearly defined cell membranes with no intercellular material (Pl. 1, fig. 5). Mitochondria and endoplasmic reticulum are sparse. β -cytomembranes are not seen. The notochord sheath contains microfibrils which are cut mainly transversely in a cross section and often appear continuous with the basal layer of the cell membrane of the notochord cells. There is no outer lining membrane to this sheath and the microfibrils do not penetrate between the mesenchymal cells of the perichordal tube.

Fifteen-day stage

The embryos studied varied from 14.9 to 15.9 mm. c.r. length before fixation. The mesenchyme of the vertebral column now shows condensations of cells in the regions of the developing intervertebral discs (Pl. 1, fig. 2). The notochord also begins to show evidence of segmentation, its cells being more numerous in the intervertebral regions than in the sites of the developing centra. The cells are still arranged in a close epithelial pattern and there is no sign of a network formation. They contain numerous glycogen granules and droplets. Beginning segmentation is also evident in the sheath (Pl. 1, fig. 2). Although its over-all diameter is fairly uniform, the clearly staining part thins out in the regions of the centra and is here separated from the notochord cells by an interval which takes up little stain, and may even appear clear, probably due partially to shrinkage. The staining properties of the sheath are similar to those observed in the earlier stage except that the red stain with van Gieson is confined to the most peripheral parts of the sheath. Irregular purple staining with Weigert's elastin is still evident. There is no staining with the Wilder-Gomori silver impregnation. The P.A.S. reaction is also more intense in the very periphery and persists after pre-treatment of the section with saliva. Staining with Best's carmine confirms the absence of glycogen in the sheath; there is no alkaline phosphatase activity.

Examination of the notochord cells with the electron microscope shows little difference from the 13-day stage and only slight variations between the intervertebral and intravertebral regions. The main change is an increase in the degree

of vacuolization of the cells (Pl. 2, fig. 6) which, at least in part, is due to their glycogen contents. Cell membranes are again well defined (Pl. 2, figs. 6, 7). The basal cell membranes show spike-like projections into the inner part of the sheath in the intravertebral regions where two clearly defined zones can be distinguished, an inner zone of less density and an outer dense zone with irregularly arranged microfibrils (Pl. 2, figs. 6, 8). The latter corresponds to the deeply stainable part as seen in light microscopy, whilst the inner zone seems to correspond, at least in parts, to the pale interval mentioned above. In the intervertebral regions the notochord cells are associated closely with the microfibrils of the sheath, although even here there is, in places, some greater aggregation of microfibrils at the periphery (Pl. 2, fig. 7). The direction of the fibrils varies with the regions, being longitudinal in the intervertebral regions and predominantly circular in the intravertebral regions. There is no limiting membrane externally and the mesenchymal cells of the perichordal tube are closely associated with the outer part of the sheath.

Seventeen-day stage

The embryos studied varied from 18.0 to 21.2 mm. c.r. length before fixation. The notochord now shows intervertebral dilatations and intravertebral constrictions (Pl. 1, fig. 3), except in the basisphenoid and coccygeal regions where it retains its primitive cylindrical form. In the dilatations and their extensions into the intravertebral regions, there is a high degree of vacuolization in or/and between the notochord cells which gives to the whole an appearance somewhat like a 'reticulated epithelium'. Cell boundaries cannot be discerned and the impression is gained of a true syncytium. The larger vacuoles, which may be intercellular spaces, contain material which gives a positive saliva-resistant P.A.S. reaction and stains with alcian blue and mucicarmine. Glycogen is present in the form of fine intracellular granules. Only small cell remnants are left in the centre of the constrictions. The sheath is most marked and thick in the cartilaginous centra and thins out towards the dilatations almost to vanishing point where these are widest. It is no longer eosinophil, but otherwise gives staining reactions similar to those of the earlier stage. There is again a differentiation of the thick part of the sheath into a narrow, more deeply staining periphery and a wide, paler, homogeneous inner zone.

Electron micrographs of the notochord in the intervertebral regions reveal that most of the large vacuoles are, indeed, intercellular. Secondly, the notochord cells are separated by clearly defined cell membranes (Pl. 3, fig. 9). Some of the large intercellular vacuoles contain microfibrils similar to those present in the sheath. Smaller vacuoles are present inside the cytoplasm and are commonly related to strands of the endoplasmic reticulum. Mitochondria are very scanty. The sheath, about 2μ thick, shows scattered, irregularly arranged microfibrils on an otherwise empty background. There is no external limiting membrane to this sheath which, therefore, is continuous with the matrix surrounding the perichordal cells (Pl. 3, fig. 9).

The notochord sheath in the intravertebral regions is considerably thicker (about 10μ) and encloses scattered cellular debris (Pl. 3, fig. 10). Thus there is no longer a sharp demarcation, internally, of the sheath. The cellular remnants differ in size and the larger ones contain tiny vesicles (Pl. 3, fig. 11). As in the 15-day

stage, the sheath shows two zones, an inner one with few microfibrils and an outer one with a much greater condensation of microfibrils (Pl. 3, fig. 10). Again there is no external limiting membrane to the sheath.

DISCUSSION

These observations on a limited series of rabbit embryos illustrate the comparatively rapid transformation of the notochord from the primitive arrangement as a rod of cells to a stage where continuity of the cells is lost in the regions of the developing centra. In the 13-day stage, the notochord shows the primitive arrangement but by 15 days, a slight intervertebral fusiform expansion associated with intravertebral narrowing is present. By 17 days, continuity of notochord cells is lost in the developing vertebrae, concomitant with a considerable expansion in the intervertebral regions. At least three possible explanations can account for this process of segmentation. Köllicker (1879) considered the mechanism to be one of passive displacement of cells due to pressure exerted upon the notochord during the chondrification of the vertebral bodies. This view received the support of Schaffer (1910) and Dawes (1930). Our study shows that, by the 15-day stage, there is already an increase in the cell population in the intervertebral regions but no constriction of the sheath in the intravertebral regions to suggest that pressure is being exerted from outside at these sites. One alternative mechanism is localized proliferation of the intervertebral notochord cells. Mitoses have been recorded up to the 3·5 mm. stage in human embryos by Prader (1945). Although the present investigation concerns embryos far older than these, it covers the vital period when segmentation first appears, but no mitoses were seen in the notochord cells. A third possibility is migration of cells from the intravertebral to the intervertebral regions. Our material suggests that this may be the first important step in the early segmentation of the notochord but, in the progression of the segmentation, two other factors contribute, degeneration of the remaining cells in the centra and vacuolization in the intervertebral regions.

There is also segmentation of the sheath. The width of the sheath is uniform throughout the notochord in the 13-day stage but, by the 15-day stage, it shows considerable thickening in the intravertebral regions without any increase in width in the intervertebral regions. This difference between the two regions is more marked by the 17-day stage and the sheath is approximately five times thicker in the developing centra than in the regions of the intervertebral discs, where it thins out almost to vanishing point over the notochord dilatations.

The arrangement of the notochord cells is epithelial in character to begin with, which is clearly brought out by the electron micrographs. Cell boundaries are distinct and remain so, even when the appearance changes to a network pattern. There is therefore no true syncytium such as was described—justifiably, on the basis of light microscopy—by Williams (1908) and Dawes (1930), and referred to as ‘chorda reticulum’ by Peacock (1951). The transformation of the original epithelium into a network arrangement with mucoid material in the intercellular spaces is not unlike the formation of the enamel pulp.

The staining reactions of the sheath indicate that collagenous material is present in

a matrix containing acid mucopolysaccharides. The weak but definite staining with Weigert's elastin in the outer part of the sheath suggests that it contains, in addition, some elastic material. Such a composition is compatible with the finding of microfibrils on electron microscopy, although no definite periodicity of these fibrils has been demonstrated. Electron microscopy showed, furthermore, two definite zones to the sheath in the intravertebral regions in the 15- and 17-day stages, an outer, dense fibrillar and an inner 'emptier' zone corresponding to the deeply staining and the pale, more homogeneous portions of the sheath seen with the light microscope.

With regard to the origin of the sheath, no definite conclusions can be drawn from the present study. Continuity seen, on electron microscopy, between some microfibrils of the sheath and the basal cell membranes of the notochord cells in the 13-day stage would suggest a notochordal rather than a perichordal origin, a view which links up with Duncan's (1957) observation. Also the differentiation of the sheath into the two zones described supports this theory. The absence, on electron microscopy, of any definite external limiting membrane to the sheath does not necessarily negate this view. However, since the sheath is well marked even in the earliest stages examined, younger embryos will have to be investigated for the elucidation of this particular problem.

SUMMARY

1. Descriptions are given of the histology and fine structure of the notochord in rabbit embryos of 13, 15 and 17 days. Points particularly noted are the general characteristics of the notochord cells, the development of segmentation and the structure and histochemical reactions of the sheath.
2. The notochord cells show an epithelial arrangement in the early stages but later are transformed into a network pattern. Cell boundaries on electron microscopy are distinct in all three stages and there is no evidence of a true syncytium.
3. Only cell debris remains in the intravertebral regions at 17 days.
4. Histochemical reactions of the notochord sheath are described. It is composed of collagenous material in a matrix containing acid mucopolysaccharides.
5. Electron microscopy of the sheath shows the presence of microfibrils which, in the 15- and 17-day stages, are concentrated towards the periphery of the sheath.
6. There is no external limiting membrane to the sheath.
7. The mechanism of segmentation of the notochord and of the origin of the sheath are discussed.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. T.S., 13-day rabbit notochord. The notochord cells show an epithelial arrangement and the sheath is wavy in outline. Outside the sheath are mesenchymal cells forming the perichordal tube. Masson's trichrome, $\times 770$.

Fig. 2. L.S., 15-day rabbit notochord. The section passes through an intervertebral region (I.V.) and a developing centrum (C.). In the intervertebral region there is a slight dilatation of the notochord, the cells filling the sheath. In the developing centrum, the notochord cells are fewer and there is a pale interval between them and the most peripheral part of the sheath (arrowed). Azan, $\times 280$.

Fig. 3. L.S., 17-day rabbit notochord. The section passes through an intervertebral region (I.V.) and a developing centrum (C.). There is a high degree of vacuolization in or/and between the notochord cells, giving an appearance somewhat like a 'reticulated epithelium'. Only small cell remnants remain in the developing centrum. Azan, $\times 280$.

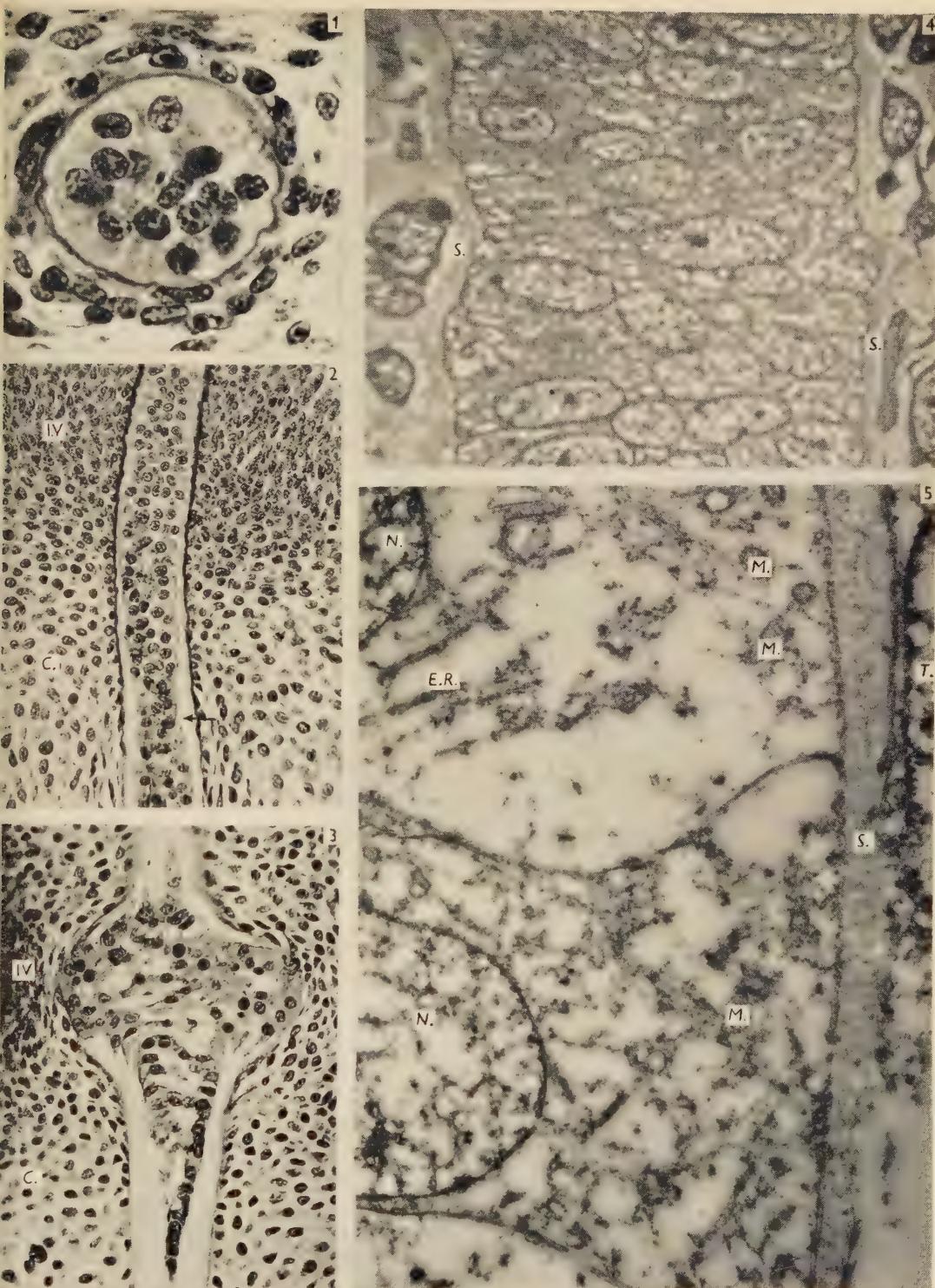
Fig. 4. L.S., 13-day rabbit notochord. Electron micrograph. The nuclei are oval in a transverse direction and the cells are arranged in an epithelial-like pattern. Small vacuoles are present in the cytoplasm. Cell boundaries are clearly seen. The sheath (S.) shows no evidence of an external limiting membrane. Palade-fixed, $\times 2100$.

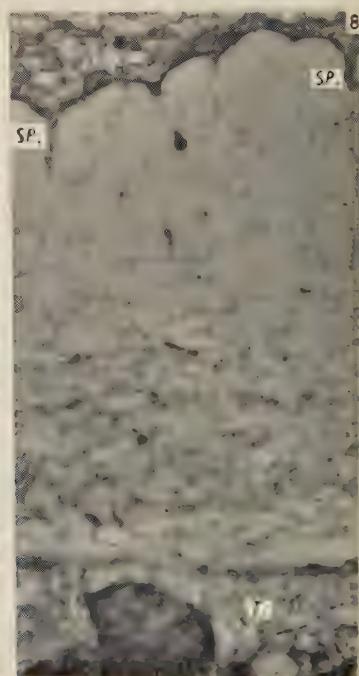
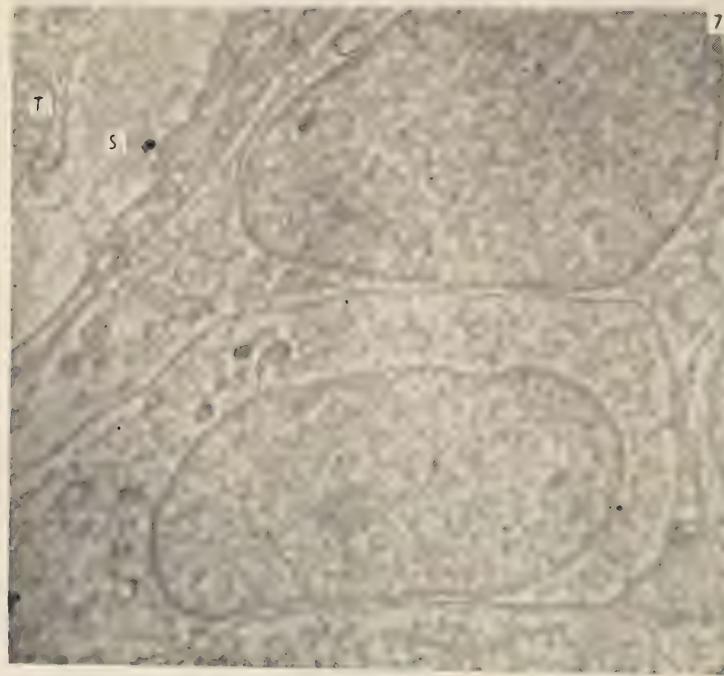
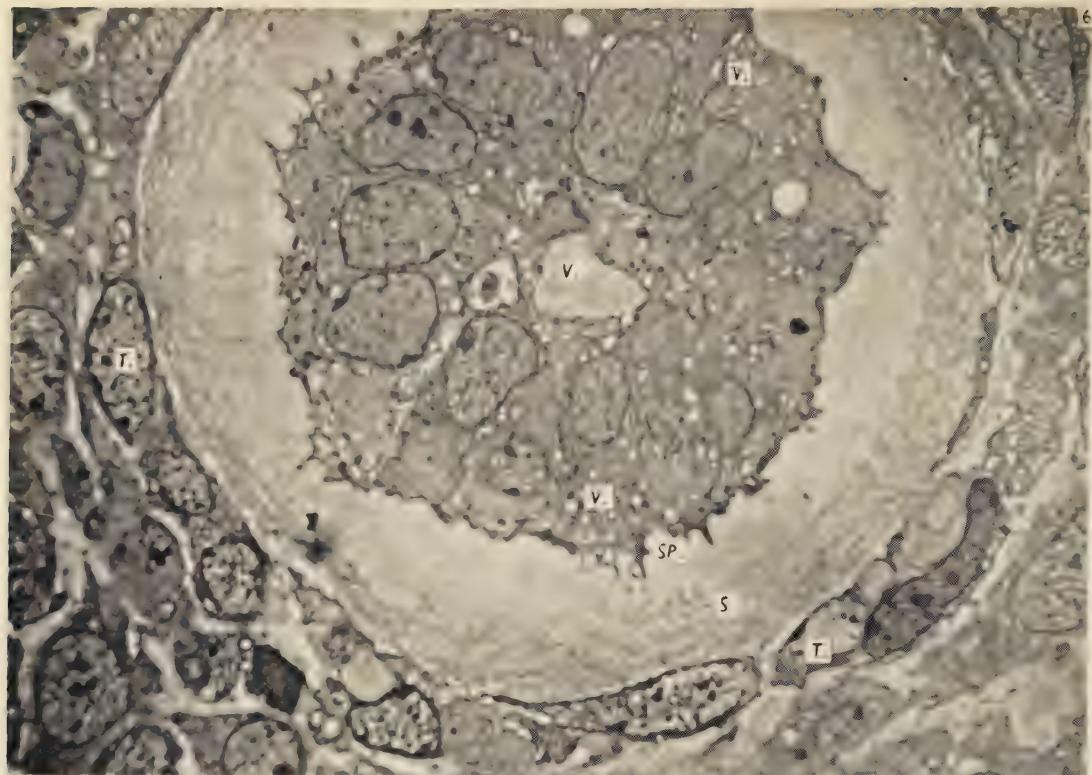
Fig. 5. Thirteen-day rabbit notochord. Electron micrograph. Peripheral parts of the notochord cells, cut obliquely, to show nuclei (N.), mitochondria (M.) and strands of endoplasmic reticulum (E.R.). Part of a cell of the perichordal tube is shown (T.). Microfibrils forming the sheath (S.) can be seen. Palade-fixed, $\times 19,000$.

PLATE 2

Electron micrographs of 15-day rabbit notochord

Fig. 6. T.S. of notochord in intravertebral region. The notochord cells show vacuoles (V.), and basal spikes (SP.) project into the sheath (S.) the outer zone of which is composed of densely arranged microfibrils. Outside the sheath are mesenchymal cells of the perichordal tube (T.). Palade-fixed, $\times 2500$.





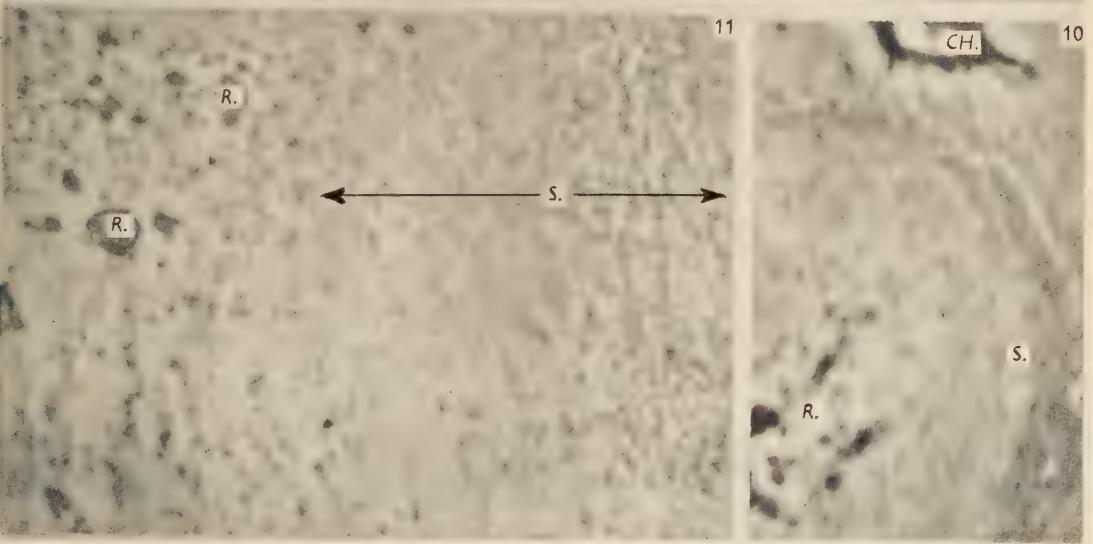
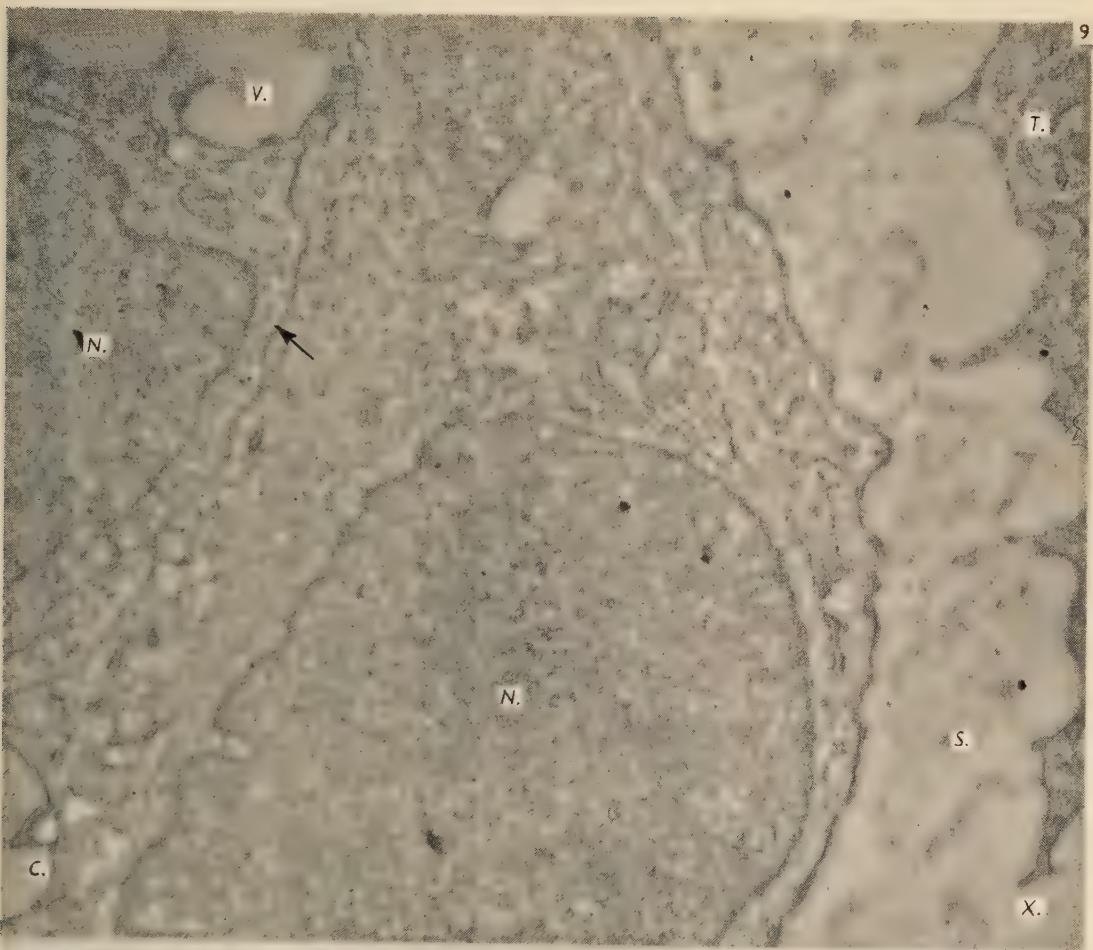


Fig. 7. Oblique section of notochord in intervertebral region. The sheath (S.) shows no clear division into zones. A cell of the perichordal tube (T.) is also seen. Dalton-fixed, $\times 8750$.

Fig. 8. L.S. of notochord sheath in intravertebral region to show basal spikes (SP.) of the cells, an inner and an outer zone to the sheath, and part of a cell of the perichordal tube (T.). Dalton-fixed, $\times 10,000$.

PLATE 3

Electron micrographs of 17-day rabbit notochord

Fig. 9. T.S. of intervertebral region. Parts of two notochord cells and their nuclei (N.) are shown, with a distinct cell membrane (arrowed) between the cells. Intercellular vacuoles (V.) are shown. Part of a perichordal cell (T.) is seen, and below it (X.), no evidence of an outer limiting membrane to the sheath (S.). Dalton-fixed, $\times 13,000$.

Fig. 10. T.S. of intravertebral region to show the general arrangement of the cell remnants (R.) and the sheath (S.). Outside the sheath there is a cartilage cell (CH.) of the developing centrum. Palade-fixed, $\times 3000$.

Fig. 11. T.S. of intravertebral region of notochord showing remnants of notochord cells (R.), and notochord sheath (S.) with well-marked inner and outer zone. Palade-fixed, $\times 8500$.

HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON
THE BLADDER AND URETER, WITH PARTICULAR
REFERENCE TO ALKALINE PHOSPHATASE
AND GOLGI MATERIAL

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A number of investigators (e.g. Gomori, 1941; Kabat & Furth, 1941; Bourne, 1943; Bern, 1949) have noted that the transitional epithelium lining the urinary tract shows a strong reaction for alkaline phosphatase, and that in some species a reaction is also given by the subepithelial connective tissue. The reaction in this type of epithelium seems to have been regarded as a diffuse one, and apart from the observation that the surface cell layer is negative (Gomori, 1941; Bourne, 1943), no details of the locus of the activity have been furnished. The reason for this may in part be due to the fact that the reaction here is rather intense, and that with the cobalt sulphide method of Gomori (1939), which was utilized in previous studies, detail is masked if the incubation period is too long (Martin & Jacoby, 1949).

In the present study the azo dye technique, as well as the cobalt sulphide technique, was employed for the histochemical demonstration of alkaline phosphatase in the bladder and ureter of laboratory animals. Results with the azo dye technique are not complicated by diffusion and give results identical with those of the cobalt sulphide method, provided the incubation period in the latter is suitably chosen.

It was observed that, with the exception of the surface layer, the majority of the cells in this type of epithelium show an intracytoplasmic reaction, confined to the cell periphery, usually in the form of an apical 'cap'.

A strictly intracytoplasmic alkaline phosphatase reaction is not very common, but in the case of the epithelium of the small intestine (Emmel, 1945; Deane & Dempsey, 1945; Novikoff, Korson & Spater, 1952) and of the large intestine in certain species (Martin, 1951), it has been found to correspond in position to that of the Golgi 'apparatus'. It seemed possible, therefore, that in the cells of transitional epithelium Golgi material might occupy the same site as that which shows the intracytoplasmic alkaline phosphatase activity.

No information could be traced, however, concerning Golgi material in transitional epithelium, not even from the very extensive literature on the Golgi 'apparatus' dealt with by Hirsch (1939) and by other reviewers of the subject (Pappenheimer, 1916; Kirkman & Severinghaus, 1938; Hibbard, 1945). A study was therefore made of the distribution of Golgi material in this type of epithelium, utilizing silver, osmic acid and sudan black techniques. The distribution was found to be similar to that of alkaline phosphatase.

Recently, Threadgold (1956) has claimed that aniline blue can be employed to demonstrate the Golgi 'apparatus', and it was found in the present work that this

dye selectively stains the same zone of the cells of transitional epithelium as was found to be osmiophil.

The possibility that either neutral red or other types of granules might be found in relation to the Golgi zone was investigated by examining with ordinary light microscopy and with phase contrast both unstained and supravitally stained cells, freshly scraped from the bladder mucosa. It was found that neutral red granules are present in the cells, but are not topographically related to the Golgi zone.

Regarding the connective tissue alkaline phosphatase reaction, it was confirmed that some species show, in addition to the epithelial reaction, a strong reaction in a band of subepithelial connective tissue, which on further study proved to be the tunica propria: no reaction was given by the submucosa.

MATERIALS AND METHODS

Specimens of bladder and ureter were removed from 13 guinea-pigs, 8 rabbits, 9 rats, 17 mice and 9 cats. The histochemical distribution of alkaline phosphatase was studied on tissues fixed in either 80 % alcohol or absolute acetone and two techniques were employed, namely, Kabat & Furth's (1941) modification of Gomori's (1939) cobalt sulphide technique, and a modification (Gomori, 1952) of Menten, Junge & Green's (1944) azo dye technique, using Na α -naphthyl phosphate as the substrate and Brentamine Fast Red TR salt (B.D.H.) as the diazonium compound. The reaction proved to be rather intense, so that the optimum incubation period with the cobalt sulphide technique was found to be rather short, usually $\frac{1}{2}$ hr. or even less, otherwise localization was masked by too heavy a deposit. Diffusion of the reaction to nuclei occurred rather rapidly, sometimes within 10 min. With the azo dye technique, diffusion to nuclei does not occur and it is only necessary to expose the sections to the substrate until a sufficient degree of colour intensity is obtained; this was about 15–20 min. for most specimens. The results with these two techniques were identical, providing the incubation period with the cobalt sulphide technique was suitably chosen.

For the demonstration of Golgi material, the modification of Kolatchew's osmic acid technique as described by Baker (1950) was employed, the tissues being divided into three pieces and osmication carried out for 3, 3½ and 4 days. Sections of mouse epididymis were used as controls to the bladder sections, and these showed the typical supranuclear osmiophil network. Aoyama's silver technique (see Baker, 1950) was also employed for the demonstration of the Golgi 'apparatus', but although control sections of mouse epididymis showed the classical network, the results with the bladder sections were inconsistent.

Baker's (1944) sudan black technique was carried out on 5 μ thick frozen sections of formal-calcium fixed material. Sections of mouse and cat small intestine were employed as controls to the bladder sections. These preparations of small intestine showed the typical supranuclear collections of lipochondria (Pl. 2, fig. 26) that were described and illustrated by Baker (1954).

Following Threadgold's (1956) claim that he was able to stain the Golgi 'apparatus' of the shell gland of the dogfish with a dilute solution of aniline blue in 90 % alcohol, trial was made with this dye in the present study. Threadgold obtained the best results by treating 70 % alcohol-fixed sections with catechol before staining, but in

the present work, the results obtained with bladder and ureter were much the same with or without catechol. Both 80 % alcohol and alcoholic Bouin proved to be suitable fixatives, and a 1 % solution of aniline blue in 90 % alcohol was found to be a satisfactory concentration of the dye. Sections of rat epididymis were used as a control, and although staining was somewhat capricious, a network was visualized, roughly of the same form and occupying the same position in the cell as that seen with the osmic acid technique for the Golgi 'apparatus'. It is not claimed, however, that this technique has wide applicability in studies on the Golgi 'apparatus'; more work would obviously be needed to determine the limits of its usefulness.

To determine whether neutral red granules are present in the cells of transitional epithelium, scrapings from the lining epithelium of the bladder of guinea-pigs, cats and mice were supravitally stained by the procedure described by Baker (1944). In addition, unstained cells were examined both with ordinary light microscopy and phase contrast. Some of these preparations were suspended in Baker's normal saline mixture, and others in the cold hypertonic mixture (30 % sucrose: 2 % sodium chloride) advocated by Dalton & Felix (1954). In the latter mixture, the cells preserve their form for longer periods.

RESULTS

Before proceeding to a description of the histochemical results, it is necessary, in view of the present findings, to draw attention to the arrangement of the subepithelial connective tissue of the bladder and ureter, since many observers fail to distinguish a tunica propria from a submucosa and not all text-books are clear in their accounts, though von Möllendorff (1930) gives a detailed and well-illustrated account of the histological features of the region.

In the present investigation, it was found that whilst in all species studied the tunica propria can be readily distinguished from the submucosa by routine methods of fixation and staining, a particularly clear differentiation is made by staining with van Gieson after 80 % alcohol fixation (Pl. 1, figs. 1-4). The tunica propria consists of a subepithelial band of closely packed fibres and is fairly sharply marked off from the more loosely and irregularly arranged coarse fibre bundles of the submucosa. A few smooth muscle bundles may lie here and there near the junction of these two fibre layers (Pl. 1, fig. 4), but this is an uncommon finding. Not only are the fibres of the two layers differently arranged, but under certain conditions they were found to exhibit different staining qualities. For example, after 80 % alcohol fixation and staining with van Gieson, the tunica propria was much more lightly stained than the submucosa in the cat and rabbit (Pl. 1, figs. 1, 2), but in the rat and mouse (Pl. 1, fig. 3) and guinea-pig (Pl. 1, fig. 4), the reverse was the case. This differential staining depends at least in part upon the fixative, since it was not found in either the cat or rabbit bladder after fixation in Heidenhain's 'Susa'.

The observation of von Möllendorff (1930) that the fibres of the tunica propria and submucosa are collagenous, was confirmed; no argyrophil fibres were demonstrated in these layers with standard silver techniques for reticulum, apart from a few that were located immediately below the lining epithelium.

The histochemical distribution of alkaline phosphatase

All species studied showed virtually the same pattern of reaction for alkaline phosphatase in the lining epithelium of the bladder and ureter. In addition, a subepithelial connective tissue reaction was present in the ureter of the rabbit and in the bladder of the guinea-pig, rabbit and sometimes in the cat, but not in the mouse or rat, the reaction being sharply confined to the tunica propria.

(a) *The bladder.* In the guinea-pig bladder (Pl. 1, fig. 5), the reaction in the tunica propria was intense and appeared after shorter incubation periods (i.e. about 5 min. with the cobalt sulphide technique) than did the reaction in the transitional epithelium. The epithelium showed a moderately intense reaction, mainly in the deeper and middle cell layers. No reaction was seen in the surface layer, and if a reaction was present in the cells of one or more layers subjacent to it, it was usually rather weak. The cell reaction was entirely intracytoplasmic and confined to the periphery of the cell, either in the form of an apical 'cap', or less commonly as a complete peripheral reaction.

The rabbit bladder (Pl. 1, fig. 10) also showed a strong reaction in the tunica propria, which forms a wider band than in the guinea-pig. The reaction in the epithelium was not a strong one, and was confined to the periphery of the cell cytoplasm of the deeper cell layers. Most specimens did not show well-marked reaction 'caps', but a thin peripheral zone of reaction, completely or almost completely outlining the cells.

The cat bladder presented some difficulty with regard to precise localization of alkaline phosphatase. The tunica propria, which forms a wide band in this species, gave a weak patchy reaction in some but not all specimens. The reaction in the lining epithelium was also found to be patchy, so that whilst some parts were strongly positive within $\frac{1}{2}$ hr. of incubation with the cobalt sulphide technique, other parts remained negative even after prolonged incubation. Diffusion of the reaction with this technique occurred rather readily, so that the positive areas were soon diffusely blackened. This, together with the fact that the epithelial cells are relatively small in this species, made localization at the cellular level difficult. Results with the azo dye technique were more satisfactory, because of the absence of diffusion. It was found that by incubating the sections for about 40 min. instead of the usual 20 min. employed for the other species, a clearly defined reaction was brought out in most but not all parts of the section. The pattern of the reaction was then seen to be the same as in the other species, i.e. the surface cell layer was negative, but in the cells deep to it a reaction was present and confined to the periphery of the cell cytoplasm, often in the form of an apical 'cap' (Pl. 2, fig. 17).

The results obtained for the bladder of the mouse (Pl. 1, fig. 13) and the rat (Pl. 2, fig. 14) were identical. In the specimens examined from both species, the transitional epithelium lining the bladder had only 2-3 layers of cells, compared with 5 layers in the other species. Some of the surface cells were somewhat flat but others were tall, with filamentous or 'flame'-like free surfaces. The nuclei of the tall cells, especially in the mouse, were particularly large, measuring as much as $20\ \mu$ in diameter. No reaction for alkaline phosphatase was given by the tunica propria of either species, but the lining epithelium gave a particularly strong reaction. The reaction usually appeared within a 10 min. incubation period with the cobalt

sulphide technique, but no reaction appeared in the surface cell layer, even after prolonged incubation. In the two deeper cell layers the reaction was confined to the periphery of the cell cytoplasm, often in the form of an apical 'cap'.

(b) *The ureter.* The reaction given by the lining epithelium of the ureter was the same in all species studied, but a reaction was given by the tunica propria in the rabbit only: none of the guinea-pig specimens showed a tunica propria reaction, even though this tissue reacted strongly in the bladder.

There are more layers of lining epithelial cells in the ureter than in the bladder; in the guinea-pig, rabbit and cat there are about 8 layers, compared with 5 in the bladder, and in the mouse and rat there are about 5 layers, compared with 2-3 in the bladder.

The reaction in the lining epithelium was essentially the same as in the bladder, and was best marked in the middle cell layers; the surface cell layer, and sometimes one or more cell layers below it, showed no reaction, whilst in the deeper layers the reaction was rather weak. The reaction was confined to the peripheral part of the cytoplasm of the cells in the form of apical 'caps', and these were particularly well defined in the ureter, where the cells are rather elongated with pointed apices (see Pl. 2, fig. 20, for rabbit; figs. 21 and 22 for guinea-pig; fig. 23 for rat).

In addition to the epithelial reaction, two other sites of enzyme activity were present in the bladder and ureter of the rat. As is well known to be the case with other rat tissues, many of the smaller blood vessels gave a reaction in the tunica intima, and a fibre reaction was seen in the adventitia of some arteries and also in fibres around the smooth muscle cells of the muscle coats of the bladder. By means of this reaction, the subepithelial capillary plexus is very clearly demonstrated (Pl. 2, fig. 23).

The distribution of osmiophil and argyrophil material

With the osmic acid technique for the demonstration of Golgi material, it was found in all the species that the lining epithelial cells of both bladder (Pl. 1, figs. 6, 11; Pl. 2, figs. 15, 18) and ureter (Pl. 2, fig. 25) contain osmiophil material at the periphery of the cell cytoplasm, generally in the form of an apical 'cap'. The localization of this material in each species was almost identical with the localization of the reaction for alkaline phosphatase. No osmiophil material was seen in the surface cell layer, and in some instances, the osmiophil 'caps' were best marked in the middle cell layers (see Pl. 1, fig. 6; Pl. 2, fig. 25). Some preparations showed in addition a few osmiophil perinuclear granules in a proportion of the cells.

The results with Aoyama's silver technique for demonstrating the Golgi 'apparatus', were not very satisfactory. In some sections, apical 'caps' were impregnated, similar to those seen in osmic preparations, but this was not a consistent finding; even in the same section other appearances were commonly encountered, for example, filaments and granules scattered irregularly in the cell cytoplasm, and sometimes a basal row of granules was present in the surface cell layer.

The distribution of sudanophil material

Frozen sections of the bladder of all the species, when stained with sudan black, showed the presence of sudanophil material (stained blue to blue-black) in the

lining epithelial cells, and as in the case of alkaline phosphatase and osmiophil material, it was localized at the periphery of the cell cytoplasm, either occupying the entire cell circumference or the apical region only, in the form of a 'cap'. The 'caps', however, were usually somewhat thinner than those seen in osmic acid and alkaline phosphatase preparations. The surface cell layer itself contained no sudanophil material, whilst the peripheral staining of the deeper cell layers was rather faint (Pl. 1, figs. 7, 12; Pl. 2, figs. 16, 19). In some specimens, a few sudanophil perinuclear granules (lipochondria) were present in a proportion of the cells. No staining of the cells occurred with sudan IV.

Staining with aniline blue

It was found that after staining sections of the bladder and ureter with aniline blue, in all the species the peripheral or apical region of the cytoplasm of the majority of the lining epithelial cells, except those of the surface layer, was selectively stained, and the 'cap'-like stained zone was similar in form to the osmiophil zone (compare figs. 24 and 25 of Pl. 2).

Studies on freshly isolated cells

Examination of fresh scrapings of bladder mucosa of the guinea-pig, mouse and cat, supravitally stained with neutral red, revealed a ring of neutral red granules in the epithelial cells, lying in a perinuclear position. Usually, the ring of granules appeared complete, but in some cells it was crescentic (Pl. 1, fig. 8). Some isolated cells of the surface layer were seen and these contained but a few tiny granules or none; these detached surface cells preserved the indented shape of their lower surfaces, which are apposed to the domed apices of the pyriform cells.

Unstained epithelial cells, freshly scraped from the bladder mucosa and examined with phase contrast microscopy, showed a perinuclear ring of granules of varying sizes in the same part of the cell as the granules seen in neutral red preparations. This region was separated by a pale zone from the peripheral part of the cytoplasm, which appeared dark and finely granular (Pl. 1, fig. 9). The pale zone can often be clearly recognized in fixed and stained preparations of the bladder of all the species studied, whether paraffin embedded or cut frozen (see, for example, fig. 7 of Pl. 1).

DISCUSSION

This study of the histochemical localization of alkaline phosphatase in the bladder and ureter has confirmed that two main sites of activity are present in these tissues, namely, in a subepithelial band of connective tissue, which proved to be the tunica propria, and in the cells composing the transitional epithelium.

A tunica propria, as distinct from a submucosa, is readily distinguished in the five species studied, not only by the arrangement of its fibres, but in certain circumstances by its staining qualities, which indicates that the fibres of the two regions may have some differences in chemical composition. This is confirmed by the fact that in some species (e.g. guinea-pig, rabbit and sometimes the cat), the fibres of the tunica propria of the bladder give a strong reaction for alkaline phosphatase whereas those of the submucosa do not. What function the enzyme has in this site is not

clear, but a connective tissue reaction is by no means uncommon in other tissues. Other fibre reactions seen in the present study were in the adventitia of rat arterioles and in a fibrous network lying between the smooth muscle cells of the rat bladder and ureter. This peculiarity of rat smooth muscle was observed in a previous study on the large intestine (Martin, 1951).

In the cells of the lining epithelium of the bladder and ureter of all species studied, the enzyme was found to be sharply localized to the peripheral or apical part of the cell cytoplasm, except in the cells of the surface layer and sometimes in one or more layers deep to it. This finding would indicate that this region of the cell is a specialized zone, and evidence that such is the case was provided by the fact that with the osmic acid technique for demonstrating Golgi material, the same zone of the cell was strongly osmophil, and although the results with Aoyama's silver technique were not consistent, argyrophil 'caps' were sometimes demonstrated in the cells. Furthermore, it was found that this special zone of the cells stained selectively with aniline blue and was sudanophil with sudan black but not with sudan IV.

In view of these results, it is probably justifiable to regard the apical or peripheral part of these cells as the Golgi zone. The form and location of the material demonstrated is unusual for a vertebrate somatic cell, but the findings were consistent for the five species investigated, and furthermore, other tissues used as controls to the bladder sections in the various techniques gave results which are generally accepted as typical for those tissues, i.e. mouse epididymis showed a supranuclear Golgi network with both the osmic acid and silver techniques, and sections of mouse and cat small intestine showed supranuclear collections of lipochondria with the sudan black technique, similar to those illustrated by Baker (1954).

It is appreciated that in using the terms Golgi 'material', 'body' or 'apparatus', one signifies no more than the appearance produced when osmium or silver is reduced from solution and adsorbed in a particular part of the cell when certain well defined techniques are followed. No inference can be drawn as to the chemical constitution of the material causing the reduction of these heavy metals (see Hibbard, 1945; Baker, 1953). Even though there is evidence from chemical studies that phospholipid is a constituent of Golgi material (Baker, 1944; Schneider & Kuff, 1954), it does not follow that this is the reason that osmic acid is reduced in the Golgi zone. There has indeed been endless dispute as to the form and nature of the material referred to as the Golgi 'body' or 'apparatus'. Many investigators have claimed that the structure demonstrated by the classical osmium and silver techniques is an artifact. For example, Baker (1944) and Thomas (1948) came to the conclusion that the classical 'apparatus' is formed by the progressive deposition of osmium or silver in the interspaces between lipoid-covered neutral red vacuoles. They considered these vacuoles to be the essential elements of the Golgi system. In later works, Baker (1949, 1954) used sudan black to demonstrate what he then believed to be the Golgi lipid, and found that small bodies, which he called lipochondria, are present in the same region of the cell as that impregnated with osmium. He believed that osmium deposits around the lipochondria, which may be simple or vacuolated, to form the classical network. Baker ceased to use the term 'neutral red vacuole' in these later works, since he found (Baker, 1949) that the vacuoles of the region do not invariably have an affinity for neutral red, and also because the

dye, especially in higher concentrations, may give rise to vacuoles where none existed before (see also Hirsch, 1939). Recently, Lacy (1955a) has claimed that this phenomenon is due to the production of large numbers of lipoidal bodies which then segregate the dye.

However, there is an accumulating body of evidence that the so-called Golgi 'apparatus' is a genuine intra-cellular structure, having much the same form as that indicated by the classical techniques, and occupying the same position in the cell. Also, there are recent observations which support the old view that the 'apparatus' is canalicular or vesicular, and that it has no topographical relationship to lipochondria or neutral red vacuoles. Much of this recent work has been carried out on mammalian tissues which show a well-defined Golgi network with the classical techniques. For example, Simpson (1941), studying free-hand sections of frozen dried epididymis, duodenum, pancreas and nerve cells, claimed that the 'apparatus' can be seen as a coiled tubular structure, and is not topographically related to neutral red bodies. Bensley (1951) also supports the view that the 'apparatus' is canalicular or vesicular, and he suggests that the vesicles contain a watery solution.

From studies on the duodenum and epididymis (Dalton & Felix, 1953), epididymis only (Dalton & Felix, 1954), and pancreas (Lacy, 1954, 1955b) it has been found that the Golgi 'apparatus', essentially in its classical netlike form, can be easily recognized in fresh unfixed cells: it appears dark with phase-contrast microscopy (Dalton & Felix, 1953), and is an entity quite distinct from neutral red bodies or lipochondria (Dalton & Felix, 1953, 1954; Lacy, 1954, 1955a). In electron microscope studies on the duodenum and epididymis (Dalton & Felix, 1953, 1954) and pancreas (Lacy & Challice, 1956), the 'apparatus' was seen to consist of a system of vacuoles, outlined by double membranes, and it was also found in these studies that both osmium (Dalton & Felix, 1953, 1954) and silver (Lacy & Challice, 1956) was deposited in the region of the double Golgi membranes when the tissues were treated by classical methods for the demonstration of the Golgi 'apparatus'.

The results of the present study on the cells of transitional epithelium have not shown any evidence that the Golgi material is arranged in the form of a network in these particular cells, but indicate that it is arranged as a diffuse peripheral accumulation. Even examination of freshly isolated cells by phase contrast has not revealed any network, the peripheral part of the cytoplasm appearing uniformly dark, but containing some fine scattered granules.

The fact that the peripheral part of the cytoplasm of these cells is stained with sudan black is interesting, since it seems to be unusual for this dye to stain any intracellular structure except the lipochondria. Lacy (1955b) reports that the Golgi 'apparatus' of pancreatic cells is not stained by the dye, and Moussa (1956) finds that it does not stain the 'apparatus' of peptic cells. On the other hand, Dalton & Felix (1954) found that in the epididymis, a component of the Golgi 'apparatus' does stain with sudan black and that the 70% alcohol in which the dye is dissolved removes some of the Golgi material. In this context, it is worthy of note that the depth of the zone stained by sudan black in the cells of transitional epithelium was usually less than that shown by osmium preparations, aniline blue, or by the alkaline phosphatase reaction.

Regarding the relationship of neutral red granules to the peripheral (Golgi) zone in these cells, it was clear that the granules lie in a perinuclear ring and are not spatially related to the Golgi zone. It is surprising, if these granules or those seen with phase contrast are lipochondria, that so few were evident as stained bodies in Sudan black preparations.

It is not possible at present to offer an explanation for the presence of alkaline phosphatase in the cells of transitional epithelium, since so little is yet known either of the functional significance of the enzyme or of possible absorptive or excretory activity of the epithelium. The fact that there is usually less enzyme activity in the more superficial cell layers and none in the surface cell layer, and also that the osmiophil material is often similarly diminished, may indicate an ageing of the more superficial cells and prove an observation of interest in studies on the function and replacement of these cells.

SUMMARY

1. A histochemical study of the bladder and ureter has shown that in the five species investigated (guinea-pig, rabbit, rat, mouse and cat), the reaction for alkaline phosphatase is localized in the peripheral part of the cytoplasm of the lining epithelial cells, usually in the form of an apical 'cap'. The cells of the surface layer, however, are consistently negative.

2. The fibres of the tunica propria of the bladder give a strong reaction for alkaline phosphatase in the guinea-pig and rabbit, and sometimes a patchy reaction is seen in the cat, but no reaction is seen in the rat or mouse. The fibres of the submucosa do not react in any of these species.

3. Osmiophil material is localized in the same part of the cell cytoplasm that gives a reaction for alkaline phosphatase, and this part of the cell also stains with Sudan black (but not with Sudan IV), and with aniline blue. It is suggested that this special zone of the cell is the Golgi zone.

4. Neutral red granules are demonstrable in cells freshly isolated from the bladder mucosa; they lie in a perinuclear position, and are not spatially related to the Golgi zone. With phase contrast, the Golgi zone appears dark and finely granular.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Cat, bladder. Stained with van Gieson after 80 % alcohol fixation. Note that the tunica propria (*T.P.*) is composed of a subepithelial band of closely packed fibres which are not so intensely stained as those of the submucosa. $\times 84$.

Fig. 2. Rabbit, bladder. Stained with van Gieson after 80 % alcohol fixation. The tunica propria (*T.P.*) is composed of closely packed fibres which are not so intensely stained as those of the submucosa. $\times 94$.

Fig. 3. Mouse, bladder. Stained with van Gieson after 80 % alcohol fixation. In this species, the closely packed fibres of the tunica propria (*T.P.*) are more deeply stained than those of the submucosa. $\times 112$.

Fig. 4. Guinea-pig, bladder. Stained with van Gieson after 80 % alcohol fixation. The closely packed fibres of the tunica propria (*T.P.*) are more deeply stained than those of the submucosa and in places are separated from them by bundles of smooth muscle fibres (*M*). $\times 112$.

Fig. 5. Guinea-pig, bladder. Alkaline phosphatase; azo dye preparation. Note strong reaction in the tunica propria. In the epithelium, the reaction is confined to the apical parts of the cells in the form of 'caps'. The cells of the surface layer are negative and those just deep to them are negative or weakly positive. $\times 310$.

Fig. 6. Guinea-pig, bladder. Osmic acid preparation for Golgi material. Note that the osmiophil material, like the alkaline phosphatase reaction (fig. 5), is confined to the apical parts of the cells in the form of 'caps'. $\times 310$.

Fig. 7. Guinea-pig, bladder. Frozen section; sudan black preparation. Peripheral part of cell cytoplasm is stained, the stained area having the appearance of a thin apical 'cap'. Surface cell layer and deeper cell layers are unstained. $\times 310$.

Fig. 8. Guinea-pig, bladder. Group of freshly isolated epithelial cells, supravitally stained with neutral red. The neutral red granules are arranged in a perinuclear ring or crescent, whilst the peripheral part of the cell is almost free of such granules. $\times 520$.

Fig. 9. Guinea-pig, bladder. Freshly isolated unstained cell; phase contrast. Note the dark and granular perinuclear zone, separated by a light zone from the dark peripheral part of the cytoplasm, which is finely granular. Compare with preceding preparation (fig. 8).

Fig. 10. Rabbit, bladder. Alkaline phosphatase; azo dye preparation. Note strong reaction in tunica propria. The cells of the deeper layers of the epithelium show a narrow peripheral zone of reaction, in some cases in the form of an apical 'cap'. $\times 280$.

Fig. 11. Rabbit, bladder. Osmic acid preparation for Golgi material. Osmiophil material is located at the periphery of the cells, except those of the surface layer, and is often in the form of an apical 'cap'. $\times 280$.

Fig. 12. Rabbit, bladder. Frozen section; sudan black preparation. Sudanophil material is confined to the periphery of the cells, often in the form of an apical 'cap'. The cells of the surface layer are unstained. $\times 280$.

Fig. 13. Mouse, bladder. Alkaline phosphatase; azo dye preparation. Note only 2-3 layers of epithelial cells. No reaction in cells of the surface layer, but in those of the deeper layers, a reaction is seen at the periphery of the cells, usually in the form of apical 'caps'. Tunica propria does not react in this species. $\times 310$.

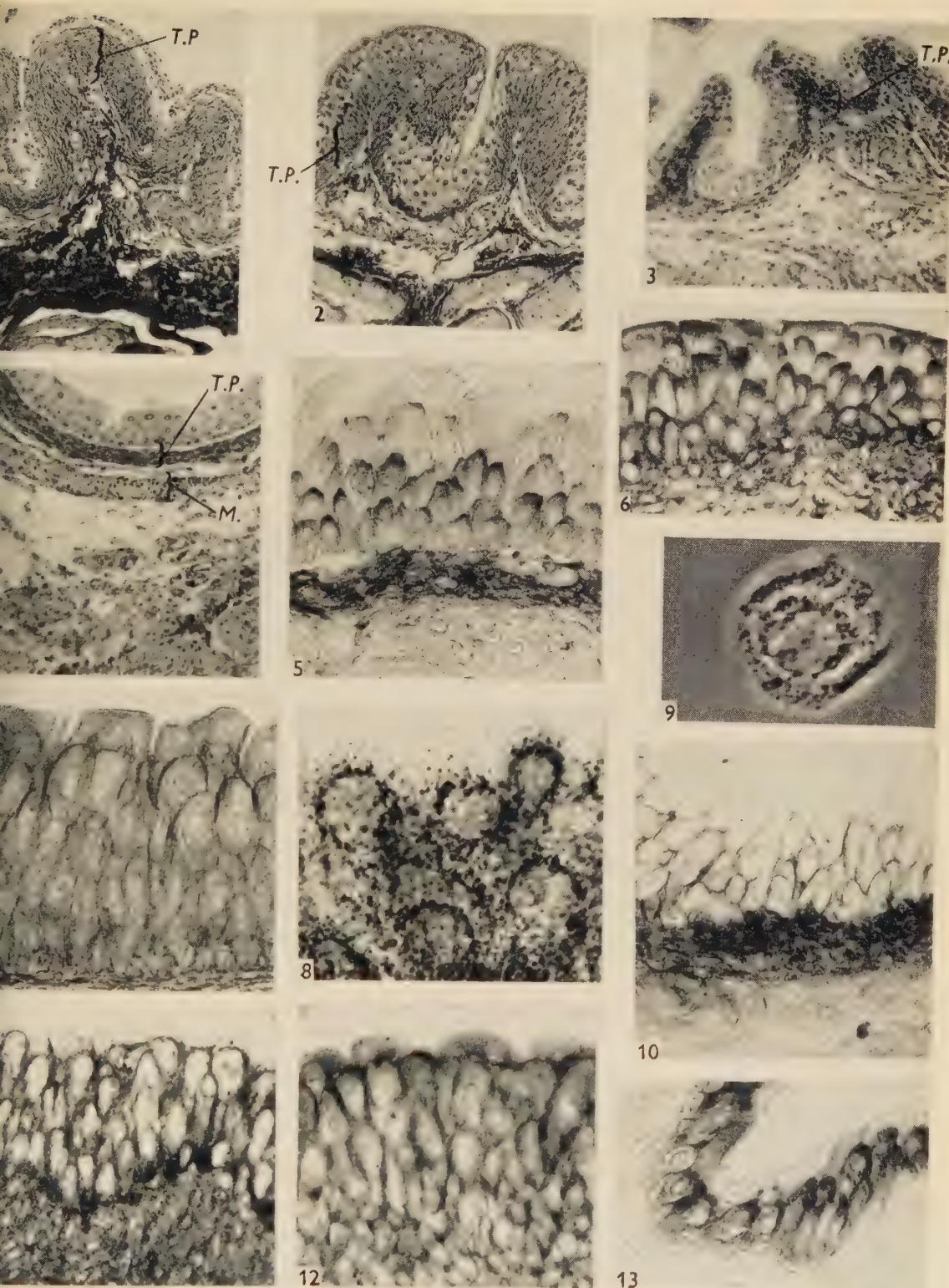
PLATE 2

Fig. 14. Rat, bladder. Alkaline phosphatase; azo dye preparation. Note only 2-3 layers of lining epithelial cells. Pattern of reaction is the same as in the mouse (fig. 13). $\times 440$.

Fig. 15. Rat, bladder. Osmic acid preparation for Golgi material. Osmiophil material is located at the periphery of the cells, except those of the surface layer, and is often in the form of an apical 'cap'. Compare with alkaline phosphatase preparation (fig. 14). $\times 440$.

Fig. 16. Rat, bladder. Frozen section; sudan black preparation. Surface cell layer unstained, but sudanophil material is present at the periphery of the cells of the deeper layers, usually in the form of an apical 'cap'. $\times 440$.

Fig. 17. Cat, bladder. Alkaline phosphatase; azo dye preparation. Most of the lining epithelial cells, apart from those of the surface layer, show a peripheral reaction, usually in the form of an apical 'cap'. The tunica propria is negative in this particular specimen. $\times 440$.



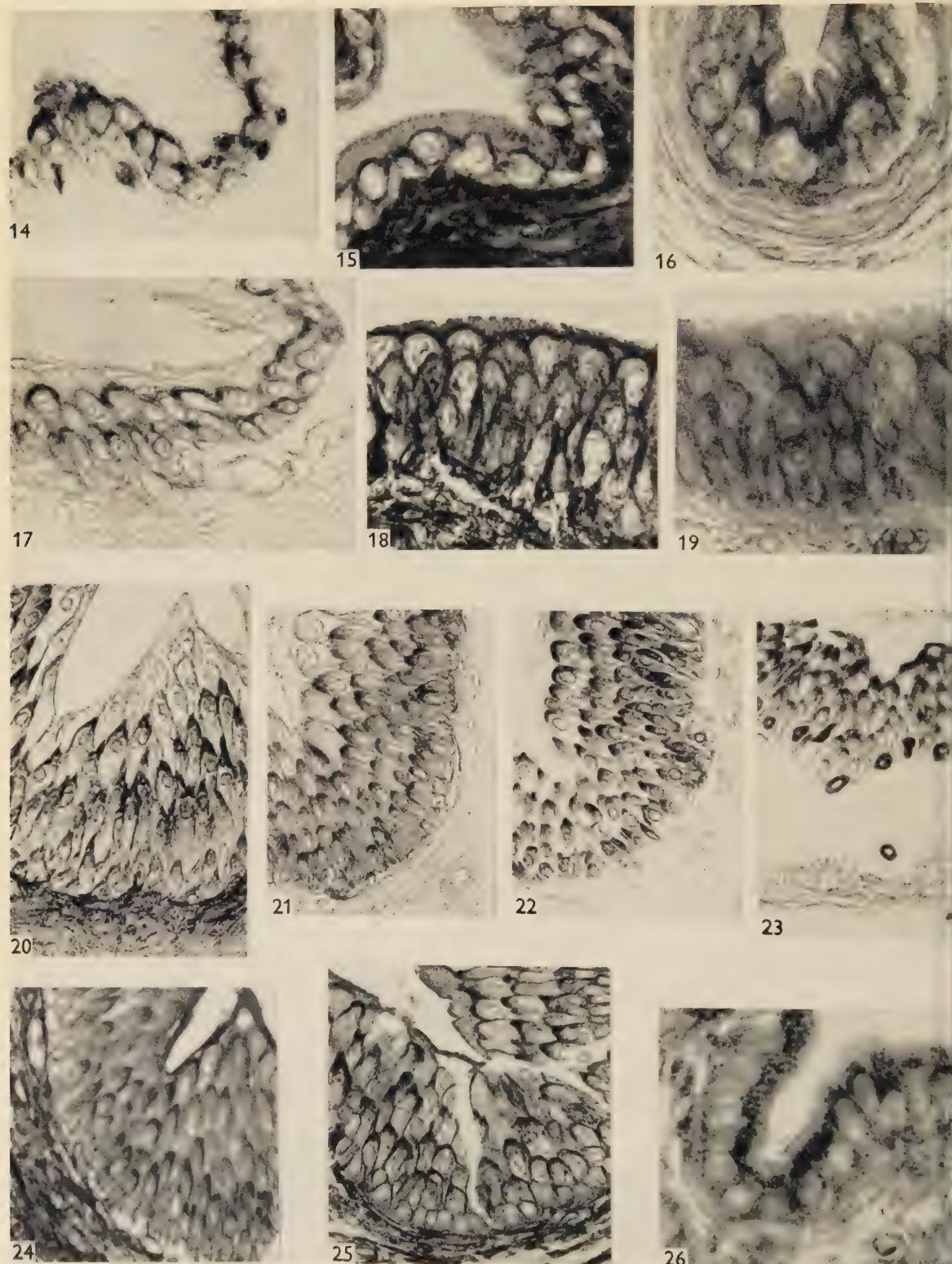


Fig. 18. Cat, bladder. Osmic acid preparation for Golgi material. Osmophil material is located at the periphery of the epithelial cells, except those of the surface layer, and is often in the form of an apical 'cap'. $\times 440$.

Fig. 19. Cat, bladder. Frozen section; sudan black preparation. Sudanophil material is present at the periphery of most of the lining epithelial cells, apart from those of the surface layer, and is usually in the form of an apical 'cap'. $\times 440$.

Fig. 20. Rabbit, ureter. Alkaline phosphatase; azo dye preparation. Note reaction in tunica propria. In the lining epithelial cells, the reaction is mainly in the cells of the middle layers, and is confined to their apical parts in the form of well-defined 'caps'. $\times 310$.

Fig. 21. Guinea-pig, ureter. Alkaline phosphatase; azo dye preparation. No reaction in tunica propria. In the lining epithelium, the reaction is confined to the apical parts of the cells, in the form of 'caps'. The surface cell layer is negative and some cells below it are negative or only weakly positive. $\times 310$.

Fig. 22. Guinea-pig, ureter. Alkaline phosphatase, cobalt sulphide preparation. Adjacent section to that illustrated in fig. 21. Reaction pattern is the same, but some diffusion of the reaction to nuclei has occurred. $\times 310$.

Fig. 23. Rat, ureter. Alkaline phosphatase; azo dye preparation. The reaction in the epithelium is confined to the apical parts of the cells in the form of 'caps'. Small blood vessels react, so that the subepithelial capillary network is well shown. A fibrous network in the muscle coats also reacts. $\times 310$.

Fig. 24. Guinea-pig, ureter. Aniline blue preparation. The apical parts of the majority of the lining epithelial cells are stained, more particularly those of the middle layers. $\times 310$.

Fig. 25. Guinea-pig, ureter. Osmic acid preparation for Golgi material. Osmophil material is present in the majority of the lining epithelial cells, except those of the surface layer, and it is confined to the apical parts of the cells in the form of well defined 'caps'. Compare with figs. 21 and 24. $\times 310$.

Fig. 26. Cat, duodenum (crypt). Frozen section; sudan black preparation. Note the lipochondria in the lining epithelial cells, occupying a supranuclear position. $\times 700$.

FURTHER HISTOCHEMICAL STUDIES ON THE PRESENCE AND NATURE OF THE GROUND SUBSTANCE OF THE CENTRAL NERVOUS SYSTEM

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Histological sections, subjected to the action of sulphuric acid with subsequent staining by azure A, reveal a metachromatic reaction in the connective tissue stroma, basement membranes, interstitial tissues, and mucous-containing cells, all of which are ordinarily orthochromatic. Kramer & Windrum (1954) have analysed this reaction and have shown that sulphuric acid treatment leads to the formation of sulphuric acid esters of carbohydrates, which stain metachromatically with azure A.

This sulphation technique has also provided another method of revealing the ground substance of the central nervous system, in addition to the periodic acid-Schiff (PAS) method used by Hess (1953). After sulphation and azure A staining, 'the intercellular matrix of the grey matter is strongly metachromatic' (Kramer & Windrum, 1954); these workers state that the metachromatic reaction corresponds in distribution to the PAS reaction.

In the present study, the observations of Kramer & Windrum (1954) have been confirmed and the effects of enzymes and further correspondence of the PAS reaction and the metachromatic reaction have been investigated in adult and newborn brains. The presence of a ground substance in the central nervous system has been confirmed and further suggestions are made as to its chemical composition.

RESULTS AND METHODS

Paraffin sections, $15\ \mu$ in thickness, of adult and new-born rat brains fixed in Rossmann's fluid were used. Rossmann fixed material was employed because previous investigations on the ground substance of the central nervous system used material fixed in this fluid. The PAS technique was applied according to the method already published (Hess, 1953).

The methods of sulphation and staining were followed according to the directions of Kramer & Windrum (1954). Slides were deparaffinized in xylene, washed in absolute alcohol and permitted to dry in air. They were then immersed in concentrated sulphuric acid for 60–75 sec. Some sections were not treated with the acid. The slides were then washed in running tap water and stained with 0·1% azure A in 30% alcohol for 5 min., rinsed in water and 70% alcohol, treated with absolute alcohol (two changes of 5 min. each), cleared in xylene and mounted. Most of the sections remained adherent notwithstanding the immersion in sulphuric acid.

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The enzymes employed were testicular hyaluronidase and pectinase. 0.018 g. of hyaluronidase were dissolved in 50 ml. of a 0.85% solution of sodium chloride. Slides were immersed in this for 24 hr. at 37° C. A 0.5% solution of pectinase was used. The pectinase was prepared according to the directions of McManus & Saunders (1950). The solution was adjusted to pH4 with sodium acetate-acetic acid buffer and slides were immersed in it for 48 hr. at 37° C. On removal from the solution, the slides were washed thoroughly in running tap water, dehydrated, washed in absolute alcohol, and allowed to dry. They were then sulphated and stained as described previously. Other slides were sulphated and then placed in the enzyme solutions. With combined sulphuric acid and enzyme treatment, some of the sections usually floated off the slides. They were then treated like frozen sections and placed into the various fluids with a glass rod.

RESULTS

Sulphation. The results of sulphation and subsequent azure A staining are essentially as described by Kramer & Windrum (1954). The violet metachromatic material is distributed between the cells. Usually the cell bodies of the neurones are not stained. Their large unstained dendrites can be seen ascending to the surface of the cerebral cortex embedded in the metachromatic material. In some places, the nerve cells are coloured green. Neurones are never stained metachromatically. The neuroglial cells and fibres also appear unstained. Further attention will be given to this point below. The tracts of the brain are stained green. The green colour of nerve cells and fibres is probably due to the orthochromatic blue coloration of azure A imposed on the yellow colour of the Rossmann fixative. The walls of blood vessels are stained metachromatically. The distribution of the metachromatic material coincides with that of the PAS-positive material in the brain (Hess, 1953). Control sections untreated with sulphuric acid essentially do not stain, probably because of the dilute stain used and the short time that it was allowed to act. However, in some places, the nerve cells are green. The green colour induced in the fibre bundles after sulphation indicates that the orthochromatic reaction of the white fibres of the central nervous system was enhanced after sulphuric acid treatment.

Hyaluronidase-sulphation. Hyaluronidase had no effect on the subsequent PAS reaction of the cerebral cortex (Hess, 1953). To determine the effects of hyaluronidase on the subsequent sulphation-metachromatic reaction, sections were incubated in hyaluronidase and the sulphation-metachromatic reaction performed. The metachromatic material is still present in the cerebral cortex, although the reaction is not as intense as in sections without hyaluronidase treatment. This may well be due to the rigorous treatment accorded the sections, rather than to any specific action of the enzyme itself. The tracts of the brain are still stained green.

Sulphation-hyaluronidase. In other sections, sulphuric acid treatment was followed by incubation in hyaluronidase to see if the sulphated form of the carbohydrates was more susceptible to the enzyme treatment. Here again the metachromatic reaction is not as intense as that of sulphated sections not subjected to hyaluronidase. However the metachromatic material is still present and the reaction is as intense as that in the sections subjected to hyaluronidase before sulphation. Again, fibre bundles are stained green.

Pectinase-sulphation. Sections subjected to pectinase incubation and then sulphated and stained reveal in the cerebral cortex metachromatic material with a distribution similar to that of sections without enzyme treatment. Tracts are stained green. However, the metachromatic reaction is much weaker than in sections not treated with pectinase or in those treated with hyaluronidase. This is perhaps an indication that the metachromatic material in the brain would be removed with incubation times longer than 48 hr.

Sulphation-pectinase. When sections are first treated with sulphuric acid and then subjected to pectinase action, the metachromatic material is removed. After staining, the sections are colourless in some places and only light green in colour in other places. The light green colour is distributed between the cells and indicates that the formerly metachromatic material has been removed and that the remaining material stains orthochromatically with the azure A, the yellow colour of the fixative contributing to the resulting light green stain. The nerve cells are unstained; the white fibre bundles assume a light green coloration.

New-born animal. That the metachromatic material and the PAS-positive material correspond can be seen after staining the brain of new-born rats. The brain of the new-born rat does not reveal any PAS reaction between the nerve cells. Sections from such a PAS-negative brain were sulphated and stained with azure A. No metachromatic material is revealed between the cells. Only a light green coloration is seen in the brain; cells are unstained. The walls of blood vessels are metachromatic. The brain of the new-born animal was left in the skull and sections made of the entire head. Other structures in the same section, such as reticulin fibres of skin and muscle, yield a metachromatic reaction.

DISCUSSION

The observation that the intercellular matrix of the brain stains metachromatically after sulphation taken together with the evidence of Kramer & Windrum (1954) of the histochemical significance of this reaction, indicates that a ground substance of a carbohydrate nature is present between the cells of the brain, and that this substance reacts with sulphuric acid to produce sulphuric acid esters of carbohydrates which are stained metachromatically by azure A.

The nerve cell processes after PAS staining or the sulphation-metachromatic reaction can be seen to issue unstained from the unstained or infrequently orthochromatically stained cell bodies, embedded in the PAS-positive or metachromatic ground substance. Thus, nerve cells and their processes are not responsible for the colours in the brain after the PAS or sulphation-metachromatic reactions.

There appears to be ample evidence that neuroglial processes are not at all involved in the PAS reaction or the metachromatic reaction of the ground substance. Tracts are PAS-negative and the neuroglia present in the white matter does not stain by the PAS method. The sulphation-metachromatic reaction after Rossmann fixation causes a green staining of some nerve cells and of the white matter, probably due to azure A blue staining imposed on the yellow colour of the fixative. Neurones and fibre tracts do not stain metachromatically. Many extracellular substances and interstitial tissues stain metachromatically after sulphation. Kramer & Windrum (1954) have described reticulin fibres that stain in this way. In our

material, the metachromatic reaction of reticulin fibres wrapping around muscle fibres or those of glands in the dermis of the skin is readily apparent. On the other hand, no metachromatic stain of neuroglial fibres, such as those wrapping around the myelinated fibres of the brain, is seen.

The sulphation-metachromatic reaction in the new-born rat brain provides further evidence that the PAS reaction and the metachromatic reaction are not due to neuroglial fibres. There is no PAS reaction and no metachromatic reaction between the nerve cells of the new-born rat brain. Yet, as is well known, some neuroglial cells and fibres are already present in the brain of the rat at birth.

The ground substance of the central nervous system is affected by enzymes in a manner similar to the basement membrane in other parts of the body, such as that in the renal tubules studied by Kramer & Windrum (1954). Hyaluronidase, they found, had no effect on the capacity of basement membrane to stain metachromatically. The same may be said of the effects of hyaluronidase on the ground substance of the brain. Hyaluronidase also has no effect on the subsequent PAS reaction of the brain (Hess, 1953). It thus appears that the ground substance of the central nervous system is not hyaluronic acid. Kramer & Windrum (1954) showed that an incubation in pectinase of under 60 hr. was not successful in preventing the appearance of metachromasia after sulphation of the renal tubular basement membrane. Similarly, in the present material, 48 hr. incubation in pectinase did not succeed in preventing metachromasia of the ground substance of the central nervous system after sulphation, although the metachromatic reaction was weakened considerably. However, the metachromatic material of the renal tubular basement membrane was removed in 18 hr. if the sections were sulphated before enzyme treatment. At the end of 48 hr. incubation in pectinase after sulphation, the metachromatic reaction of the ground substance of the central nervous system was absent. As Kramer & Windrum (1954) say, the 'effect might be due to partial degradation of the substrate by the reagents rendering it more labile'. Nevertheless, it does indicate that in both the basement membrane of the kidney tubules and the ground substance of the central nervous system, the sulphuric acid esters of carbohydrates are more readily attacked by pectinase than the carbohydrates themselves.

The PAS reaction and the sulphation-metachromatic reactions appear to correspond very closely in their revelation of the ground substance of the central nervous system. Kramer & Windrum (1954) believe that 'some of the metachromatic elements seen in the central nervous system after sulphation... represent sulphated lipid'. However, Hess (1953) showed that various lipid solvents, including hot methanol-chloroform, had no effect on the subsequent PAS reaction of the ground substance of the central nervous system. It is doubtful, therefore, if lipids are responsible for the metachromasia after the sulphation-metachromatic reaction.

Wyckoff & Young (1956), on the basis of their electron micrographs, believe that 'most of the material between the formed elements (of the brain) contains mitochondria and is therefore cellular protoplasm'. They suggest that this material might be neuroglial processes. The discussion above indicates that neuroglia is not responsible for the staining reactions of the ground substance. Thick paraffin sections, usually of $15\ \mu$, have been used for most of the studies on the ground substance of the central nervous system. An even thicker paraffin section yields a more

intense staining reaction of the ground substance. A thinner section of $5\ \mu$ produces a less intense reaction. It appears that the spaces between the cells and fibrils in the neuropil of the cerebral cortex in which the ground substance rests are very small ones. A thick section includes many of these labyrinthine spaces and hence yields an intense staining reaction. A thin section includes less of the small intercellular space and hence yields a faint staining reaction. The ultra-thin sections employed in electron microscopy include only a minute amount of intercellular space and matrix. Thus, very thin sections and high resolution are needed to resolve the space containing ground substance, a space which may well consist only of the small interval between two membranes at the high magnification afforded by the electron microscope. The evidence that the ground substance is not lipid indicates that this substance does not necessarily have to appear black in the electron microscope after fixation with osmic acid. Electron micrographs of higher resolution than those employed by Wyckoff & Young (1956) are necessary before the presence of a ground substance in the central nervous system can be denied.

SUMMARY AND CONCLUSIONS

The sulphation-metachromatic reaction of Kramer & Windrum (1954) was used to study the presence and nature of the ground substance of the central nervous system in paraffin sections of adult and new-born rat brains fixed in Rossmann's fluid. A violet metachromatic reaction occurs after sulphation between the predominantly unstained cell bodies and their processes in the cerebral cortex; fibre bundles and some nerve cells stain green (orthochromatic blue by azure A imposed on the yellow colour of the fixative). The metachromatic material has the same disposition as the periodic acid-Schiff substance in the cerebral cortex. Hyaluronidase, before or after sulphation, does not remove the metachromatic material. The metachromatic material is not hyaluronic acid. Pectinase before sulphation does not remove the metachromatic material, although the metachromatic reaction is weakened considerably. However, pectinase after sulphation succeeds in removing the metachromatic material. The brain of a new-born rat that yields a negative periodic acid-Schiff reaction between the cell bodies of the cerebral cortex, also does not reveal any metachromatic material between the cell bodies after the sulphation-metachromatic reaction. A discussion is presented showing that nerve cell bodies and their processes and neuroglial cells and their processes are not involved in the sulphation-metachromatic or the periodic acid-Schiff reactions. The material yielding these reactions is the ground substance of the central nervous system. This substance reacts similarly during enzyme treatment to basement membranes in other parts of the body. The ground substance is not lipid. A discussion of the possibilities of revealing the ground substance of the central nervous system by the electron microscope is presented.

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SOME FEATURES OF THE DUCTUS ARTERIOSUS

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The ductus arteriosus connects the pulmonary and aortic trunks. In later foetal life these latter vessels have predominantly 'elastic artery' structure. The ductus was not considered by early workers (Rokitansky, 1844; Pfeifer, 1902) to differ appreciably from them in its fine structure. It is now generally agreed however that it does differ, particularly as regards its elastic tissue (von Hayek, 1935) which may be so scanty that it can be regarded as a 'muscular' artery (Boyd, 1941).

Normally the ductus closes after birth. The belief that this closure occurs within minutes of delivery (Barclay, Franklin & Prichard, 1944) has recently been disputed by Dawes, Mott & Widdicombe (1955), who found that in lambs a patent ductus persists for many hours after birth, a finding also reported in dogs by Everett & Johnson (1951). There has long been doubt also regarding the factors leading to closure. Melka (1926) suggested that it was due to pressure from the expanded pulmonary trunk and aorta, combined with the presence of intimal swellings and folds; while Swensson (1939) considered muscular contraction to be important in the process. Barcroft, Kennedy & Mason (1938) reported experiments which indicated a possible neuromuscular mechanism, and Boyd (1941) described nerve endings in the ductus wall. Kennedy & Clark (1942) considered respiration, and especially blood oxygen level to be of significance. More data was brought forward on these aspects by Born, Dawes, Mott & Rennick (1956).

A histochemical difference between the tissues of the ductus and those of the great vessels which it connects was recently reported (R. L. Holmes, 1957). This work has since been amplified, and extended to include human material.

MATERIAL AND METHODS

The greater part of the work has been carried out on rabbits. Does were killed 27–28 days after mating by an overdose of Nembutal and the foetuses immediately removed. Their thoracic and abdominal cavities were opened, and whole animals fixed in cold 10% formol-saline for approximately 18 hr. After dissection frozen sections of the ductus, pulmonary trunk and aorta were cut in various planes and mounted on clean dry slides. A number of preparations fixed as above or in Carnoy's fluid were embedded in paraffin and sectioned serially.

Similar material was obtained from a number of 9-week guinea-pig foetuses, and three human hysterotomy specimens (9, 11·5 and 14 cm. C.R. length). Some rodent preparations were treated as whole mounts, being slit open and flattened on clean slides and allowed to dry sufficiently for adherence. These, together with sectioned material, were incubated in the substrates for cholinesterase demonstration as

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described by Coupland & Holmes (1957). Other techniques included toluidin blue and Alcian blue staining for ground substances, and the PAS reaction. The techniques followed were as given by Pearse (1953). Paraffin material was stained by haematoxylin and eosin, Mallory's phosphotungstic acid haematoxylin, or aldehyde-fuchsin (Gomori) for elastic tissue; some series were impregnated by the Holmes silver technique (W. Holmes, 1947) for nerve fibre demonstration.

OBSERVATIONS

The structure of the ductus arteriosus will not be considered in detail here except in so far as this may bear on the other findings described. Examination of sections has largely confirmed the descriptions of previous workers, such as Jager & Wollenman (1942) for the human, and Kennedy & Clark (1941) for the guinea-pig.

Aldehyde-fuchsin gives a brilliant stain of the elastic tissue of the vessel walls. Such preparations show that in none of the three forms is the ductus wall lacking in elastic tissue, although its amount and disposition differ from what is found in the aorta and pulmonary arteries. A thick internal elastic lamina is present, often split into several layers. Throughout the media there are concentrically arranged laminae of elastic tissue, with much finer elastic fibrils running irregularly between them. These laminae are considerably thinner than those in the wall of the aorta or pulmonary trunk (Pl. 1, figs. 1, 2), but tend in some cases to become coarser towards the adventitia. Comparison of sections stained by aldehyde-fuchsin with those stained by Weigert's or Sheridan's stain shows essentially the same picture.

With phosphotungstic acid haematoxylin, the only part of the elastic tissue in the ductus wall to show clearly is the internal lamina, whereas all the aortic lamellae show well, probably on account of their greater thickness.

The smooth muscle of the ductus and the aorta differs in its arrangement relative to the vessel lumen. In the aorta it is disposed in well-defined inner circular and thinner outer longitudinal layers. The ductus, however, has a thin inner longitudinal layer, a thick circular layer outside this, and a variable thin outermost longitudinal layer. The ductus wall is usually thicker than that of the descending aorta, and its smooth muscle cells appear larger and less closely packed; their nuclei too are often larger and show intranuclear detail more clearly. Particularly in the guinea-pig, the ductus wall is considerably looser in structure than the aorta, and small clefts within the muscle layers are commonly seen.

Staining with Alcian blue was slight or negative for both aorta and ductus. Toluidine blue however showed narrow bands of metachromatic material in both vessels, which were most marked just deep to the endothelial surface of the ductus.

Cholinesterase activity

Transverse sections of the ductus in all three forms showed both true and pseudo (non-specific) cholinesterase activity in nerve fibres. These formed a mesh around the periphery of the section, and by counterstaining could be shown to lie in the adventitia. Finer fibres from this adventitial complex extended into the outer muscle zone of the media, but in this type of preparation no evidence of fibres reaching as far as the intima was found. The remaining tissues of the ductus wall

were all negative with respect to both true and pseudo cholinesterase activity (Pl. 1, figs. 3, 5).

Cross-sections of the aorta and pulmonary trunk also showed cholinesterase activity in nerve fibres, distributed as in the ductus. The media of these vessels, however, also gave a positive reaction (Pl. 1, figs. 3, 4). This was prevented both by incubation in a substrate containing 3×10^{-6} M eserine, and by pretreatment for 20 min. with a 10^{-6} M solution of DFP. This indicates that the positive reaction was due to pseudo cholinesterase.

This contrast between a positive reaction of the media in the aorta and pulmonary trunk, and a negative in that of the ductus was found in both rodent and human material. In the rodent sections the contrast was clearly evident after incubation in substrates of pH 5.6, but for human material a pH of 6.2 was required.

Whole thickness preparations

The entire ductus arteriosus of a number of guinea-pig and rabbit foetuses, and the adjoining portions of the pulmonary trunk and aorta, were incubated in butyryl thiocholine substrate. Such preparations demonstrated two features on microscopic examination:

(a) As might be anticipated from the above description of sectioned material, the zone of the ductus wall showed a negative cholinesterase reaction, while the walls of the other vessels were positive. Thus the ductus appeared as a light region, passing at either extremity into the dark positive zones of the pulmonary trunk and aorta respectively (pl. 1, fig. 6). The transition was not sharp, due to some overlap of negative ductus tissue and positive adjoining elements, which can be well seen in a longitudinal section through the region (Pl. 1, fig. 4).

(b) The second feature was a network of elements showing a positive cholinesterase reaction which could be clearly seen through the thickness of the ductus wall. This consisted of branching fibres ranging from about 25μ in thickness down to very fine filaments (Pl. 1, fig. 6). The whole formed a rich, though somewhat unevenly disposed, mesh. Both in size and general pattern it corresponded to a net of branching nerve fibres, such as was seen in sectioned material to lie mainly in the adventitial region. As the cholinesterase-positive zones of the preparations were approached, the net became less dense, but some branches could be seen passing into the positive zones, although here they became somewhat difficult to follow.

Silver-impregnated material

Serial paraffin sections of the rabbit ductus, cut transversely, showed nerve strands lying in the adventitia, and fine fibres passing into the media, where they appeared to end by branching. Most fibres seemed to lie in the outer layers of the muscle; a few penetrated further towards the intimal surface. The appearances corresponded to the figures published by Boyd (1941).

Some material, however, was sectioned in a plane parallel to the long axis of the ductus, so that a number of tangential sections through the wall were obtained. Some of these demonstrated a more extensive branching of fine nerve fibres, often with closely associated nuclei, probably 'sheath' in character (Pl. 1, fig. 7). Such branched structures were most evident in the region of the junction between

adventitia and media, but some, consisting of more delicate fibres, could be found in sections passing nearer to the intima. Thick myelinated fibres terminating as complex branched endings were not observed in any of these preparations.

DISCUSSION

The re-examination of the histological structure of the ductus arteriosus in these three mammalian forms generally confirms views which are already well known. It may perhaps be noted, however, that although the ductus is often thought of as an almost entirely muscular vessel, it does contain a considerable amount of elastic tissue. Indeed, although only the internal lamina reaches the size found in the aorta, the whole media shows concentrically arranged elastic lamellae. As Bory (1909) described, elastic tissue does tend to be limited to the intimal region in less mature foetuses. The pulmonary trunk and aorta in foetal life do not show the paucity of muscular elements found in adult forms.

A further point of distinction between the ductus and those two trunks which it unites is the striking absence of cholinesterase activity in the former. This feature is not the consequence of a lack of any single tissue component in the ductus which is present in the other vessels. The structural differences in all three are differences of degree, and it would appear that the enzymatic defect might well be a distinctive characteristic of the ductus smooth muscle.

As regards the nervous relations of the ductus, numerous fibres are present in the adventitia, some of which penetrate the muscle coat. Boyd (1941), among others, has considered that both thick sensory and thinner motor fibres form discrete endings in the wall. Study of tangential sections shows that the areas occupied by branching fibres are considerably larger than might appear from transverse sections, and suggests the possibility of a more extensive netlike formation.

The cholinesterase techniques, when utilized for nerve demonstration, have many of the advantages of methylene blue, and make possible the study of whole thickness preparations. Correlation between the latter, between thick sections treated by similar methods, and silver preparations suggests the possibility that the innervation of the media is by a deeper extension of fibres from an adventitial network, which form a finer terminal network among the smooth muscle cells.

As noted in the introduction, several theories of the stimulus and mechanism of ductus closure have been put forward. Born *et al.* (1956) have shown that the ductus of lambs dilates and constricts as the blood oxygen saturation falls or rises, and also that pressor amines cause a reversible constriction. Dawes *et al.* (1955) had previously demonstrated that a considerable proportion of blood flowing to the lungs might pass via the ductus in the first hours of life, and that a patent ductus might be an advantage during that period when the lungs were not fully expanded.

The role of innervation in normal ductus closure is difficult to assess. The origin of the fibres in the media is not known with certainty. The fact that the tissues of the ductus are devoid of cholinesterase activity might suggest that acetylcholine, if released locally in the ductus wall, could persist and influence its smooth muscle. The presence of cholinesterase would preclude such an action in the walls of the pulmonary trunk and aorta. Some such hypothesis could find support in the

observations of Barcroft *et al.* (1938) that stimulation of the peripheral cut end of the left vagus in a guinea-pig foetus caused constriction of the ductus; but the sensitivity of the ductus to a number of varying stimuli emphasizes the necessity for further work.

SUMMARY

1. The ductus arteriosus of rabbit, guinea-pig and human foetuses has been examined by histological and histochemical methods, and compared with the pulmonary trunk and aorta.
2. Cholinesterase and silver techniques show an adventitial network of nerve fibres in the ductus, with extensions, which may also be netlike in structure, into the media.
3. The tunica media of the ductus differs from that of the pulmonary trunk and aorta in showing no cholinesterase activity.
4. It is considered that this difference may be significant in the functional closure of the ductus.

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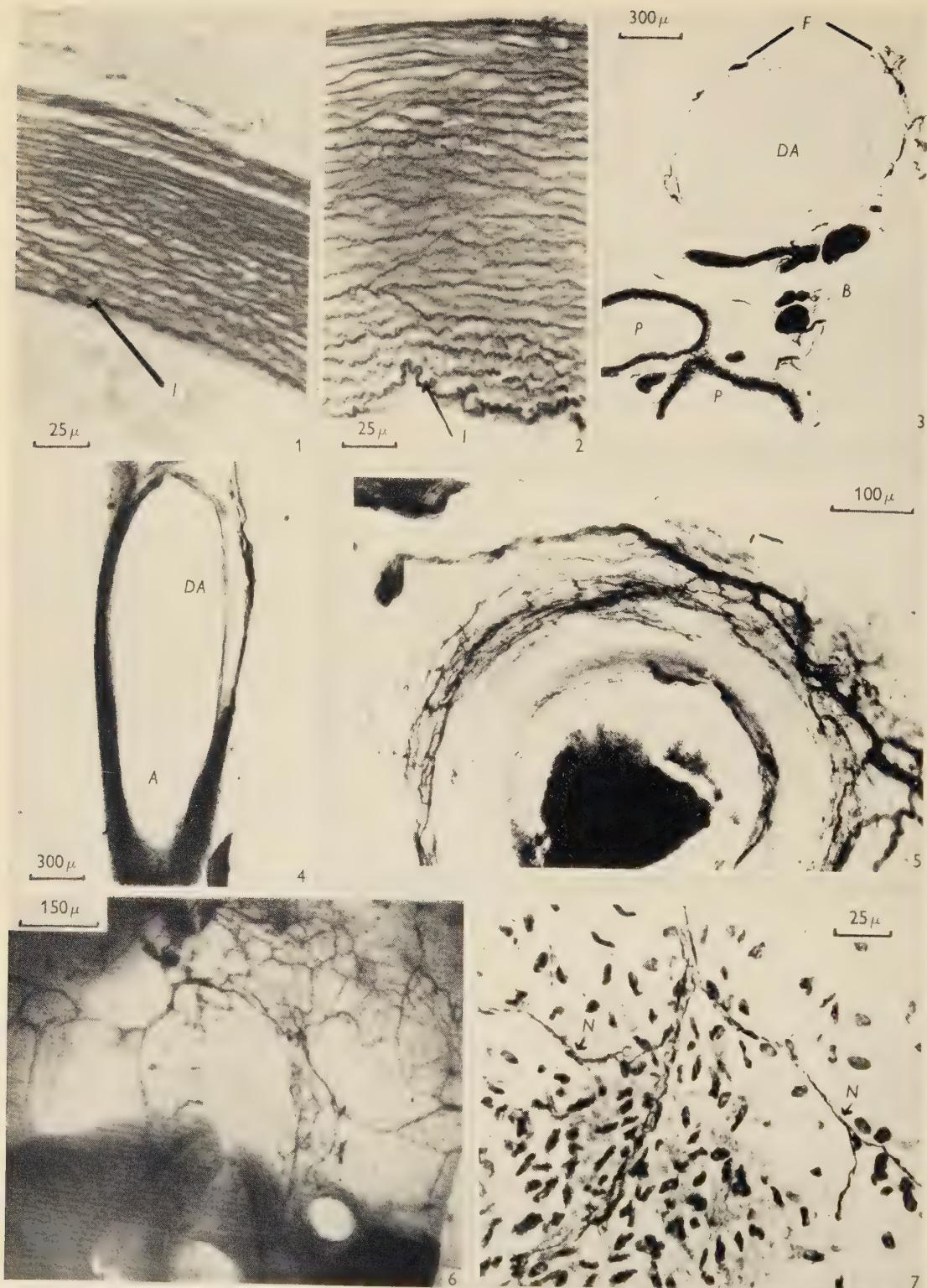
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EXPLANATION OF PLATE

All figures are from late foetal rabbit material.

Fig. 1. Transverse section of aorta, stained by Gomori's aldehyde-fuchsin. A thick internal elastic lamina (*I*) is shown, together with other laminae throughout the wall.

Fig. 2. Transverse section of the ductus arteriosus from the same animal as fig. 1, also stained with aldehyde-fuchsin. An internal elastic lamina (*I*) is present, and the rest of the wall shows thinner elastic laminae and intermediate fibres.

Fig. 3. Transverse section of the whole ductus region, incubated to show cholinesterase activity, no counterstain. The ductus (*DA*) is negative except for adventitial nerve fibres (*F*). The dark structures (*B*) below are nerve bundles and ganglia, and pulmonary arteries (*P*), both of which show a strongly positive reaction.

Fig. 4. Section through the junction of the ductus arteriosus and aorta to show cholinesterase activity. A segment of the ductus wall (*DA*) is negative and contrasts with the strongly positive aortic wall (*A*).

Fig. 5. Transverse section (200μ) of the ductus, again to show cholinesterase activity. The ductus wall is negative. Blood clot in the lumen and adherent to the vessel lining shows positive; round the periphery a strongly positive mesh of nerve fibres is present, with extensions into the media.

Fig. 6. Whole thickness preparation of the ductus and adjoining vessels, incubated for cholinesterase. The pale area is ductus wall, through which can be seen a network of nerve fibres. Below, the ductus passes into the cholinesterase-positive aorta.

Fig. 7. Tangential section (15μ) through the adventitial-medial junction of the ductus, Holmes's silver impregnation. Branching nerve fibres (*N*) are evident, which are probably part of a network.

LYMPHATICS OF THE SPLEEN

BY G. M. GOLDBERG, M.D.

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To date, the question whether deep parenchymatous lymphatics exist in the human spleen has been controversial. Ruysch (1721, quoted by Klemperer, 1938) believed that lymphatics coursed jointly with the arteries and nerves, penetrating deeply into the splenic substance. This idea was supported by Kyper (1872), who demonstrated endothelial-lined periarterial lymph vessels by injections of silver salts. Key (1861), Schweigger-Seidel (1862) and Schaffler (1922) have all drawn attention to the fact that deep parenchymatous lymphatic vessels exist within the human spleen, being connected with the Malpighian corpuscles. The presence of tumour metastases in the spleen caused Katsuki (1924, 1925) to assume the existence of deep intraparenchymatous lymphatics. On the other hand, Billroth (1861, 1862) was among those who emphatically insisted that lymph vessels never occur in the interior of the spleen. Other authors (Bailey, 1936; Drinker & Yoffey, 1941; Maximow & Bloom, 1944; Szymonowicz, 1900; Schäfer, 1885), including Kölliker (1889) and Weidenreich (1901), described finding occasional lymph vessels in the capsule and superficially situated in the thickest trabeculae, particularly in the vicinity of the hilus, but none within the red or white pulp. The majority of current text-books of histology make no mention of the deep lymphatic supply of the spleen (Klemperer, 1938; Greep, 1954; Hartman, 1930; Lubarsch, 1930; Maximow & Bloom, 1944). Some text-books state that no deep parenchymatous lymph vessels exist (Bailey, 1936; Drinker & Yoffey, 1941; Rouvière, 1938; Szymonowicz, 1900).

Previously, two carcinoma cases with splenic metastases were studied. The metastases appeared to be within a lymphatic system (Goldberg, 1957).

These findings, which suggested the presence of a deep parenchymatous vascular system related to the walls of the arterial and venous systems of the spleen, prompted the examination of a series of spleens from non-carcinomatous unselected adult necropsies.

MATERIAL AND TECHNIQUE

Sections were taken from the hilum, neighbouring spleen parenchyma and deep parenchymatous tissue. The material was fixed in formalin or Zenker's acetic acid fluid, and sections at $8\ \mu$ were made from paraffin blocks. The sections were stained with haematoxylin and eosin, Weigert's elastica counterstained by van Gieson's stain. Thirty cases were examined.

As lymphatics in the spleen are more prominent when containing epithelial mucous-producing cells, a further study was performed in two cases of metastatic carcinoma in adults, one originating in the female breast and the second in the common bile duct of a male patient (Goldberg, 1957).

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In these cases serial sections were made of the gastro splenic omentum including the splenic artery and veins as far as the hilum of the spleen, neighbouring spleen parenchyma and deep parenchymatous tissue.

The material was submitted to the aforementioned fixing and staining techniques. Colloidal iron-acid ferrocyanide (Rinehart & Abul-Haj, 1951) stain was used to demonstrate mucous secreting tumour cells.

FINDINGS—CARCINOMA CASES

Vessels were seen in the adventitia of the splenic artery in the omental tract. These vessels were distended and engorged with carcinoma cells. The tributary veins of the spleen had similar vessels containing tumour cells located subintimally. The walls of these vessels consisted of a single layer of endothelial cells, with a diameter many times that of a hemic capillary.

In the walls of the hilar arteries and of veins situated within the splenic trabeculae, tumour cells were seen (Pl. 1, fig. 1). As before, the tumour cells were in vessels in the innermost part of the adventitia, while in the veins the tumour cells were in vessels located subintimally (Pl. 1, fig. 2; Pl. 2, fig. 4).

These tumour-containing vessels were traced into the trabeculae and deeper parenchyma and were always found accompanying arteries and veins. No such vessels were found in trabeculae where arteries and veins could not be seen; neither were any tumour cells seen outside of these vessels. The vessels themselves were of a strikingly irregular calibre, being dilated in some segments and extremely narrow in others. Extremely dilated and extremely narrow segments were often found close to each other. In the arteries the vessels were always seen in the adventitia (Pl. 1, fig. 1; Pl. 2, figs. 4, 5), and in the veins these vessels were always seen subintimally, occasionally even protruding into the lumen (Pl. 1, fig. 3).

In one histological section a trabecular artery was traced along its course until it finally terminated as a follicular arteriole outside the trabecule, and within the white pulp. Vessels containing tumour cells were found along its course as far as the Malpighian corpuscle (Pl. 2, fig. 4).

In the Malpighian corpuscle tumour-containing vessels were found around the central follicular artery; these vessels were always seen to consist of a single layer of endothelium (Pl. 2, figs. 5-7).

FINDINGS—NON-CARCINOMATOUS CASES

Small subintimal spaces, with lymphoid elements in them, were found in the walls of veins of different calibre (Pl. 3, figs. 8, 9). Occasionally it appeared that the fine intimal endothelial lining bulged into the lumen by the presence of lymphoid elements (Pl. 3, figs. 10-12). These spaces always followed the same subintimal venous location, their calibre varying in neighbouring positions. The adventitia of arteries contained very narrow slits with solitary lymphoid elements in them. These minimal findings could be seen in arteries of various calibre. In the arterioles, however, significant spaces with single-layered endothelial linings were constantly observed between the white pulp and the arteriolar wall (Pl. 3, fig. 13). The spaces contained lymphoid elements.

COMMENT AND DISCUSSION

Although deep parenchymatous lymphatics have been demonstrated in the spleens of various animals (Bannwarth, 1891, quoted by Klemperer, 1938; Katsuki, 1925; Snook, 1946; Tomsa, 1863, quoted by Hartman, 1930) by means of vital staining, silver salts, Indian ink and cinnabar injections, it has not as yet been possible to visualize a deep lymphatic system in the human spleen either experimentally or by post-mortem studies.

In the carcinomatous cases studied, the spread of mucous-producing carcinoma cells permitted the visualization of a system of vessels in the walls of the splenic artery and veins in their omental tract, in the walls of trabecular arteries and veins and around follicular arterioles in the Malpighian corpuscles. The presence of these vessels in the Malpighian corpuscles preclude the possibility of their being hemic vasa vasorum since arterioles of these calibre are known to be devoid of vasa vasorum. These vessels were of a strikingly irregular calibre, always had a single-layered wall of endothelial cells and were never seen to contain red blood cells. Their subintimal location in the trabecular veins and adventitial location in the trabecular arteries was a constant feature. As these vessels were not seen in trabeculae devoid of blood vessels and were in constant relation to the blood vessels, it would seem that they were an integral part of the wall of the blood vessel.

Tumour cells were not seen in the lumen of an artery or outside a vessel or in the sinusoids of the red pulp.

In the non-carcinomatous spleens, a network of endothelial lined spaces were found in the walls of the arteries and veins. These spaces contained lymphoid elements and were situated in a similar location to the vessels described in the carcinoma cases (Goldberg, 1957).

Lymphatics not containing carcinoma cells and consisting of a single-layered endothelial wall would hardly be expected to be discernible in the adventitia of splenic arteries, because of compression by surrounding dense connective tissue. However, they are clearly visible when not compressed by such tissue, when in the subintimal location in veins and along arterioles of the white pulp.

Thus it could be concluded from the above findings that these vessels are a part of a deep parenchymatous lymphatic system of the human spleen.

It could not be decided whether the lymphatic system seen was afferent or efferent, but it is presumed that tumour cells reached the spleen in an afferent current.

SUMMARY

1. A study of the deep parenchymatous lymphatics of the human spleen was prompted by the previous finding of that system in two cases where metastatic carcinoma involved that organ.
2. A comparative study was performed in thirty spleens of non-carcinomatous unselected adult necropsies.
3. Fine endothelial lined spaces were observed subintimally in veins and along arterioles of the white pulp, containing only lymphoid elements.
4. The constant relation of these vessels to the walls of arteries and veins and their unique parallelism to those observed in the carcinoma cases, was stressed.

5. It is concluded that these vessels are a part of a deep parenchymatous lymphatic system existing in the human spleen.

The author wishes to acknowledge with grateful appreciation the help and advice given by Prof. H. Ungar, Head of the Department of Pathology. The author is indebted to Prof. M. Ickowicz, head of the Department of Anatomy and Histology, for helpful criticism, and to Dr M. Wolman who has also kindly criticized the manuscript. Thanks are also due to Mrs H. Weinman for the photographic work.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. A medium-sized artery with accompanying lymphatics. Tumour cells present. Haematoxylin and eosin. $\times 120$.

Fig. 2. A medium-sized vein with tumour cells in subintimal lymphatics. Haematoxylin and eosin. $\times 120$.

Fig. 3. Same as fig. 2. Subintimal lymph vessels with tumour cells, protruding into the lumen. Haematoxylin and eosin. $\times 370$.

PLATE 2

Fig. 4. Tumour cells in lymphatics, visualized by the presence of mucous secretion, follow the course of a trabecular artery which becomes a follicular arteriole. Colloidal-iron (Rinehart and Abul-Haj). $\times 120$. An orange filter was used in the photographic technique.

Fig. 5. A trabecular artery and a follicular artery with lymphatic vessels containing tumour cells. Haematoxylin and eosin. $\times 150$.

Fig. 6. A Malpighian corpuscle. Follicular artery with tumour cells in lymphatics around it. Haematoxylin and eosin. $\times 150$.

Fig. 7. Follicular artery in Malpighian corpuscle. Irregular lymphatics consisting of a single-layered endothelial wall. Tumour cells clearly visible. Van Gieson and Weigert's elastica stain. $\times 370$.

PLATE 3

Fig. 8. A splenic vein in deep parenchyma with subintimal lymphatic vessel containing lymphoid elements. Haematoxylin and eosin. $\times 450$.

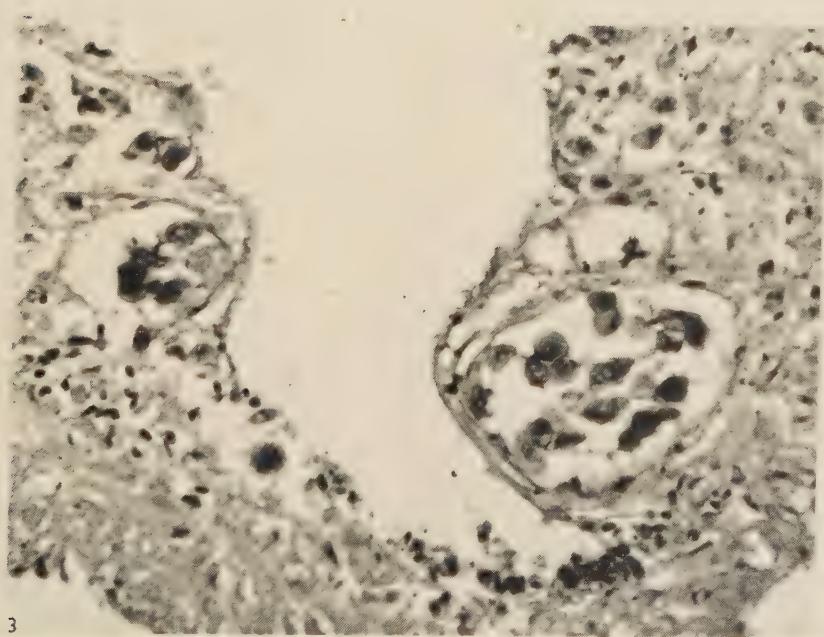
Fig. 9. As in fig. 8. Haematoxylin and eosin. $\times 360$.

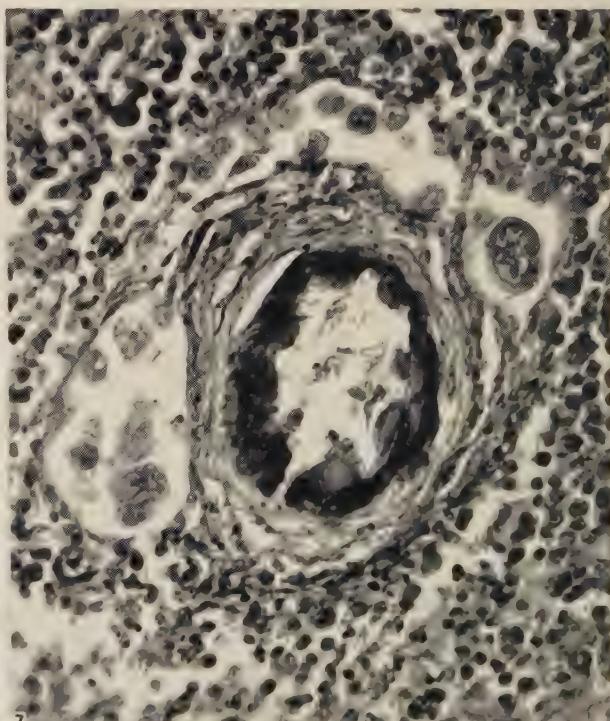
Fig. 10. A subintimal lymphatic vessel in a vein. Endothelial lining clearly visible. Plasmocyte and lymphoid element in lumen. Haematoxylin and eosin. $\times 1500$.

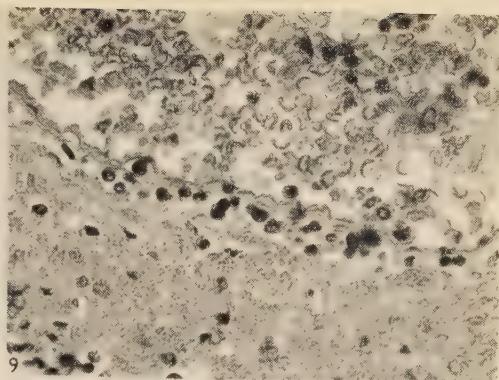
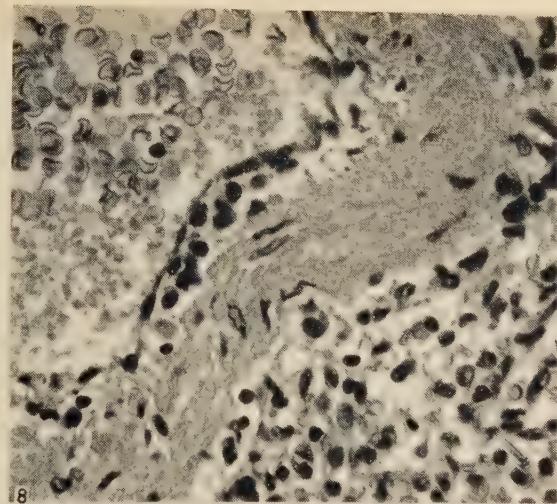
Fig. 11. A subintimal lymphatic in a vein. Endothelial lining bulging into lumen of vein. Haematoxylin and eosin. $\times 1500$.

Fig. 12. Subintimal lymphatic vessel in a vein. Engorged with lymphoid elements. Haematoxylin and eosin. $\times 1600$.

Fig. 13. A minute lymphatic vessel accompanying a splenic arteriole of the white pulp. Endothelial lining visible between arteriolar wall and lymphoid elements of the white pulp. Haematoxylin and eosin. $\times 530$.







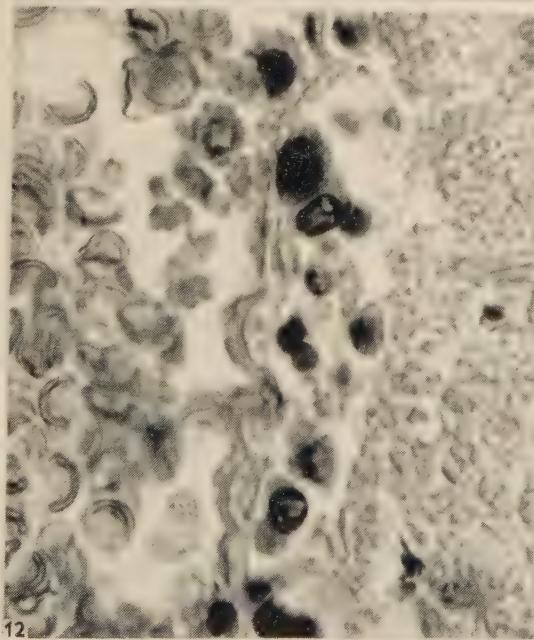
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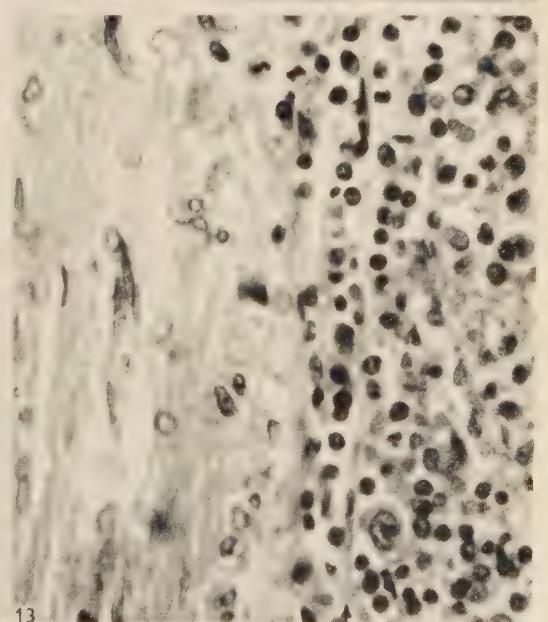
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THE 'CARRYING ANGLE' IN MAN

BY F. L. D. STEEL AND J. D. W. TOMLINSON

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Potter (1895) was the first to carry out a quantitative investigation on the obliquity between the upper arm and the fully extended and supinated forearm in man. He measured this 'carrying angle' in ninety-five men and ninety women and recorded average measurements of 173.17° and 167.35° respectively; that is to say the forearm was laterally inclined on the upper arm 6.83° in males and 12.65° in females. His measurements were carried out on the living by means of a hinged board 'applied to the limb', and also by dropping a plumb-line from the 'front and mid-line of the upper arm, the string touching the lesser tuberosity and allowed to fall exactly in the centre of the elbow'; he measured the distance between this line and the tendon of palmaris longus and derived a value for the carrying angle by elementary trigonometry. Subsequent measurements by essentially similar methods by Mall (1905), Nagel (1907) and Fick (1911) have all shown the mean female angle to be significantly greater than that of the male, though none reported a difference as great as the 5.82° recorded by Potter. In 1945, Atkinson & Elftman reported a difference between the male and female means of 1.8° , which they say is statistically significant but associated with too wide a variation to be of value in individual instances.

In order to eliminate differences due to variations in the development in the soft tissues in the arms of the two sexes it was decided to describe the vertex and arms of the carrying angle in terms of bony points, and it has been provisionally re-defined by the present authors as the acute angle lying between the tangent to the medial side of the head of the humerus produced through the tip of the medial epicondyle, and a line joining the tip of the epicondyle with the medial side of the lower end of the ulna. The tip of the epicondyle and the lower end of the ulna are points that may easily be palpated subcutaneously.

MATERIAL AND METHODS

The above subcutaneous bony points were marked with a pointer on a sheet of cardboard placed beneath the arm, the subject lying supine on the table of an X-ray machine with the forearm extended and supinated. The humeral head was then X-rayed (the plate being attached with drawing pins to the card) by means of the vertical component of a coned beam, the tube being aligned on the palpated medial



Fig. 1. Diagram illustrating 'carrying angle' defined in terms of bony points.

border of the humeral head by means of a plumb-line. The developed film having been re-pinned in its original position the two lines forming the angle were drawn on the card and the angle itself measured.

To check the accuracy of this method the angle was measured in a fresh cadaver, the body being raised into a sitting position and the arm circumducted between exposures. Four consecutive measurements gave angles of 22° , 23° , 23° and 23° .

The investigation was concerned with the left arms only of 100 Europiform adults (fifty of each sex) from staff and students of the London Hospital and Medical College. It was considered undesirable to subject any one person to more than a single X-ray exposure.

Table 1. 'Carrying angle'

	Males	Females
Mean	19.28°	18.38°
S.D.	4.67°	3.41°
Diff. of means	0.90°	
S.D. of diff.	0.82°	
Mean age (years)	23.26	21.06
Range (years)	18-40	19-30

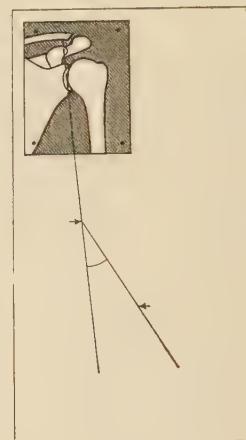


Fig. 2. Developed film re-pinned to card and 'carrying angle' drawn.

Since the carrying angle is in part due to the more distal position of the tip of the medial lip of the trochlea the 'trochlear angle' was measured in fifty dis-articulated adult humeri of known sex. The acute angle between (a) the tangent to the head of the humerus passing through the medial epicondyle, and (b) the line joining the distal parts of the lips of the trochlea, was recorded.

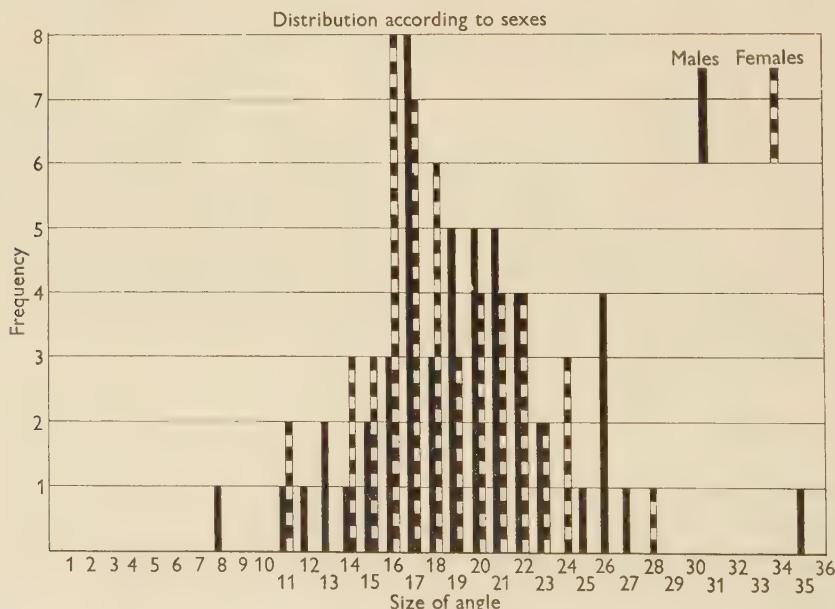


Fig. 3. Frequency histogram for the 'carrying angle'.

Table 2. 'Trochlear angle'

	Males	Females
Mean	73·5°	74·5°
S.D.	4·21°	2·95°
Diff. of means		1·0°
S.D. of diff.		1·03°

For neither the 'carrying angle' nor the 'trochlear angle' can the difference between the two groups be said to be statistically significant.

In order to find out to what extent the tangent to the head through the tip of the epicondyle is inclined to the long axis of the humerus, the latter has been provisionally defined as a line joining the mid-points of the coronal diameters of the bone at the junctions of the middle half with the upper and lower quarters. The angle between these two lines was measured in ten adult humeri and found never to exceed one degree.

Measurements of the carrying angle made by a method previously reported (Steel, 1957) on twenty adult dis-articulated human arm bones gave male and female means of 19·2° and 18·3° respectively. In view of the small sample and insignificant difference between these male and female means the authors conclude that the extremely close correlation between these figures and those obtained from living subjects is a chance finding.

SUMMARY

The human 'carrying angle' defined and measured as above appears as the parameter of a normal distribution curve.

Neither the 'carrying angle' nor the 'trochlear angle' exhibits any statistically significant difference in size between the two sexes.

We are grateful to Prof. R. J. Harrison for his encouragement and advice; to the staff and students of the Hospital and Medical College for their ready co-operation; to the Rector (the Rev. Cyril M. Armitage, M.V.O., M.A.) and Churchwardens of St Bride's Church, Fleet Street, for permission to examine the osteological material exposed during the post-war excavations, and to Mrs Moira Steel and Dr H. E. R. Chew for preparation of the drawings.

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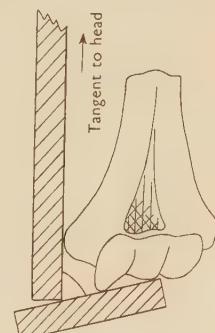


Fig. 4. Measurement of 'trochlear angle'.

REVIEWS

The Leukemias. Etiology, Pathophysiology, and Treatment. Ed. by J. W. REBUCK, F. H. BETHELL and R. W. MONTO. (£5. 4s. Od.) New York: Academic Press Inc.

This interesting volume presents the proceedings of an international symposium held in Detroit in March 1956. It contains 38 papers, with subsequent discussion, and a concluding Round-Table Discussion on Therapy.

Apart from the clinical and pathological material, there is a great deal of matter of outstanding interest for anatomists. The opening paper by Dr Marcel Bessis, on the electron microscopy of leucocytes, contains fundamental information about the structure of normal as well as of leukaemic leucocytes. The same is also true of the paper by Dr Sven Moeschlin on the phase-contrast microscopy of leucocytes. But in both instances there is very little indeed about the lymphocyte.

As might be expected, there are papers on what is now known as the 'radiation biology' of leucocytes, as also on changes undergone by leucocytes in tissue culture. Dr Albert S. Gordon and his colleagues review the problem of endocrine influences on blood formation, and Dr Craddock gives a further account of the results obtained by the recently introduced technique of leukopheresis, by means of which a new approach has been made to studying the capacity of the bone marrow to discharge cells into the circulation.

There are also speculations on the length of life of the normal lymphocyte, which on the basis of isotopic labelling techniques is estimated to be about 21 days. Speculations on the fate of the lymphocyte continue unabated, and new hypotheses are being continually launched. But the essential difference of opinion still remains, between those who think the lymphocyte is important for what it *is*, as a stem cell, or those who feel that it is of value for what it *does*.

J. M. YOFFEY

Vergleichende Neuropathologie des Menschen und der Tiere. By E. FRAUCHIGER and R. FANKHAUSER. (Pp. viii + 471; 271 illustrations. Ganzleinen, D.M. 126.60.) Berlin: Springer Verlag.

For many years veterinary pathologists have rightly deplored the lack of detailed comparative pathological studies, even of the commoner domestic animals, comparable to these readily available to workers in the field of human medicine. The handicaps under which veterinary surgeons have laboured have been due in part to the difficulties of obtaining diseased animals for examination, particularly when the disease is one that does not affect the commercial value of the carcass. In comparative pathology much confusion has also arisen from the variety of names by which the same disease may be known in different countries, or even in different parts of the same country. At the same time there is scattered throughout the veterinary literature an ever-increasing wealth of information on diseases naturally occurring or experimentally produced in animals. A reliable guide to this information cannot but be of the greatest value to pathologists and others whose field of interest is primarily in diseases that occur in man. The appearance of an authoritative work such as that of Profs. Frauchiger and Fankhauser is therefore especially welcome for, as Dr L. van Bogaert points out in his foreword, the study of diseases in animals has the great advantage that the animal can be slaughtered for pathological examination at any given stage of the disease.

In this book the authors have made a comprehensive survey of present-day knowledge of comparative neuropathology. In addition to their own painstaking investigations of nearly 3000 animals, collected over a period of twenty years, the descriptions and conclu-

sions in their book are based on an extensive review of the relevant literature. The volume is divided into General and Special Parts. The former includes a short historical survey and notes on such topics as the Neuron Theory and Neurobiotaxis. These accounts are somewhat cursory, and it may perhaps be doubted whether they are worthy of inclusion. The Special Part of the book, by far the larger part, is concerned with the pathological findings in diseases of the nervous system in animals. The chapter headings conform very largely to the generally accepted classifications of disease processes: Congenital Malformations, Metabolic Diseases, Inflammations, Tumours, etc. The book is well printed on good quality paper and the standard of reproduction of its numerous illustrations is high.

It is only to be expected that in a book of this kind there will be statements to which not everyone will subscribe; for example, many workers would not agree that the Pacchionian granulations are of importance in the absorption of the cerebrospinal fluid. Some readers might also wish to see more space given to the important findings by American veterinary workers in animals suffering from Vitamin A deficiency. These are, however, only minor criticisms made possible by the general high standard of the work.

Profs. Frauchiger and Fankhauser are to be congratulated on the production of this important work which must remain for many years the standard reference book for comparative neuropathologists. It will also be a valuable acquisition for the book-shelves of every pathologist.

J. W. MILLEN

Human Histology. A Textbook in Outline Form. By L. B. AREY. (Pp. ix + 337; 45s. 6d.) London: W. B. Saunders Company. 1957.

Many and varied are the text-books of Histology in current use. This one, as its author freely admits, is designedly without illustrations, unorthodox in treatment and written in a staccato text mostly of single-line sentences expressed with Baedeker-like concision and force.

As a summary of the verbal side of the subject it will no doubt have its uses more particularly in this country for the type of student who delights in memorizing facts, or perhaps for quick reference on some half-forgotten details which are often tedious to find in a more orthodox text. Those who already know more or less of what the book contains, by dint of long laboratory experience and familiarity with original literature, may be fascinated by the ingenuity the author displays in varying the construction of his disjointed sentences.

How very helpful a compilation of this kind would be to young research workers if it listed not the established facts, but as much as possible of the things we don't know in this ever-developing subject!

K. C. RICHARDSON

NERVE ENDINGS IN THE CONJUNCTIVA

BY D. R. OPPENHEIMER, ELISABETH PALMER AND G. WEDDELL

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INTRODUCTION

Sensory nerve fibres in the integument of the body terminate peripherally in one of two ways: either in a system of diffuse, overlapping, arborizations (commonly called free nerve endings), or in various types of compact endings (commonly called encapsulated nerve endings). As far as we know, there has never been an attempt to subdivide the diffuse type of ending into morphological subspecies; on the other hand, a morphological classification of compact endings has been undertaken by numerous workers—without, it seems, achieving any measure of general agreement on the subject.

There are, indeed, three types of compact ending which are generally recognized as distinct morphological entities. These are: (1) the basket-like formations surrounding the shafts of hairs and vibrissae; (2) the 'tactile corpuscles' of Meissner, occupying the dermal papillae underlying the papillary ridges in the glabrous areas of the hand and the foot; and (3) the deeper-lying corpuscles of Pacini, with their characteristic onion-like lamellation. Apart from these there exists an ill-defined assortment of types of compact ending, many of which have been dignified with the names of eminent anatomists. Ruffini (1906) was one of the first to point out that the taxonomic classification of these 'types' was an arbitrary and even an unprofitable exercise, since each 'type' is found to merge insensibly, through a continuous series of forms, into other 'types'. This view has received support from modern workers (see Weddell, Palmer & Pallie, 1955). On the other hand, some investigators in the field of cutaneous sensibility, seeking to discover correlations between sensory 'modalities' and morphologically recognizable nerve structures, have focused attention on certain, as it were, bands of the spectrum, and claimed to show that certain 'types' are in fact specific receptors for particular forms of cutaneous stimuli. Curiously enough it does not seem to have been appreciated that in hairy skin, which covers the greater part of the body, the only compact nerve endings are those in specific relation to hairs (see Hagen, Knoche, Sinclair & Weddell, 1953).

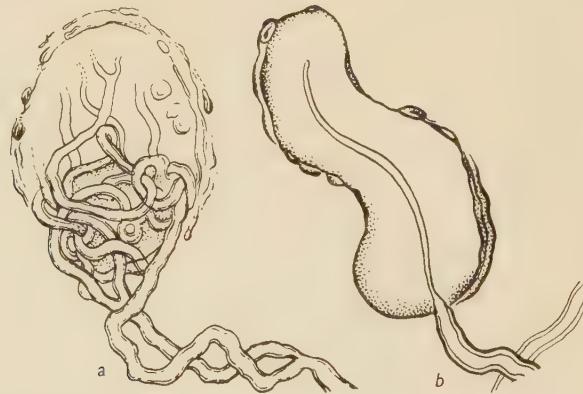
The most assiduous workers in this field were von Frey and his school, who claimed to have demonstrated that a certain type of compact nerve ending, the 'Krause end-bulb', was the specific receptor for stimulation by cold. The crucial evidence for this view was a series of experiments carried out on the conjunctiva of the living human eye, where they believed, from the reports of various nineteenth-century histologists, that 'Krause end-bulbs' were abundant. The experimental part of this work will be discussed elsewhere; at this point, it is sufficient to say that von Frey's reading of the earlier literature was, to say the least, selective. As will be shown in the ensuing section, although many workers have in the past studied the innervation of the conjunctiva, general agreement has not in fact been reached

either as to the precise morphological characteristics of a 'Krause end-bulb', or as to the regular occurrence, density and distribution of 'Krause end-bulbs' in the mammalian conjunctiva.

The present investigation was undertaken with the object: first, of discovering what lay behind the confused and contradictory state of the literature on this subject; and secondly, of finding an anatomical basis for the evaluation of experimental work on the sensibility of the eyeball. We concerned ourselves primarily with two questions: whether there is a specific morphological entity which can fairly be described as a 'Krause end-bulb', and whether 'Krause end-bulbs' or compact nerve endings of any kind are a constant feature of the conjunctiva in man and other mammals. As it happened, our findings led us to some unexpected conclusions, which will be discussed at the end of this paper.

LITERATURE

Krause (1859, 1860, 1861) was the first to describe compact nerve endings in the conjunctiva. He examined small pieces of tissue, either fresh or after maceration in dilute acetic acid, without fixation or staining. By this means he claimed to see, at the terminations of all medullated nerve fibres in the conjunctiva, characteristic 'organs' which he termed *Endkolben*. These were of two kinds. The first (Text-fig. 1a) which he found only in material from primates, was a spherical or ovoid



Text-fig. 1. Redrawn from Krause (1859) (a) end-bulb from human conjunctiva (cf. Pl. 3, figs. 31, 32); (b) end-bulb from bovine conjunctiva (cf. Pl. 1, figs. 6, 7; Pl. 2, fig. 16). Fresh, macerated specimens.

structure, 40–100 μ in diameter, with a capsule containing several layers of flattened nuclei, and served by one or more myelinated nerve fibres. Before losing their myelin sheaths and penetrating into the interior of the bulb, these fibres entered into a dense entanglement on the outside of the bulb. The second type (Text-fig. 1b) was characteristic of lower mammals, in particular the calf. Here the 'end-bulb' was several times longer than it was broad, and the nerve fibre supplying it, instead of forming an entanglement, ran directly in at one pole, losing its myelin sheath at the point of entry, and terminated in a more or less straight unmyelinated axonal

segment in the centre of the bulb. The structure as a whole was reminiscent of a Pacinian corpuscle, but without its characteristic lamellation. This simple, elongated type was also found once or twice in conjunctivae from young children.

The distribution of *Endkolben* was somewhat irregular: in what he regarded as a typical human case, however, Krause counted 76 and 87 end-bulbs respectively in strips of conjunctiva 4 mm. wide, surrounding the margins of the right and left corneae. Nine per sq.mm. was taken as a rough average for the bulbar part of the conjunctiva, rather less for other parts.

Krause observed similar structures in various other exposed mucous surfaces, including the tongue and the external genitalia. In general, these were of the rounded, complex type in man and other primates, and elongated and simple in other mammals. In addition, he described, as a rare finding in the human conjunctiva, myelinated nerve fibres which, after forming a close *Knäuel* or tangle—not, apparently, enclosed in a capsule—continued in a further uncomplicated course without losing their myelin sheaths.

Krause's main findings were quickly confirmed by H. Frey (the histologist) (1859). But in 1862 Arnold published a rebuttal of their views. Using methods similar to those of Krause on human and bovine material, he saw myelinated nerve fibres ramifying in the conjunctiva, and was even able to trace their non-myelinated offsets to the epithelium, where they seemed to end blindly. Of the structures described by Krause he saw no trace. On the other hand, after much searching he did observe a few rounded, apparently structureless excrescences on the course of myelinated nerve fibres in the vicinity of a tear in the tissue, and found subsequently that by suitably maltreating his specimens, these appearances could be reproduced. He interpreted them as being effects of damage to myelin sheaths and of the flowing-out of the substance of a torn nerve fibre into an artificial tissue space. The fact that nerves, after entering such a 'bulb', commonly re-emerged from it and ran a further course, forced him to conclude that the 'bulbs' were not true nerve endings, but technical artefacts.

In the following year, Arnold was the victim of a polemical outburst by Lüdden (1862-3), a medical student in Kölliker's laboratory. Lüdden claimed to have seen end-bulbs, such as Krause had described, in man and calf, which could not have been produced in the manner suggested by Arnold and to which Arnold's artefacts bore no resemblance. His conclusions and drawings were accepted by Kölliker and incorporated in several editions (e.g. 1889) of his text-book of histology. Arnold made a dignified reply to the attack, but thereafter seems to have lost interest in the controversy.

In 1867 Mauchle reopened the subject. Using the newly introduced gold chloride and osmium tetroxide techniques, he was unable to obtain satisfactory preparations; but by treating tissues with acetic (or still better, sulphuric) acid he obtained results which confirmed Krause's and Lüdden's observations in man and calf. He noted that the end-bulbs were not confined to the vicinity of tears or cuts, and that divided nerve fibres showed no tendency to form bulbs or blobs. Mauchle extended his observations to rabbits, cats, pigs, dogs, rats and mice; but in none of these could he find end-bulbs. He confirmed Arnold's finding of free nerve endings, and differed from Krause's view that all myelinated fibres terminated in end-bulbs.

In the same year, Lightbody (1867), using caustic potash, acetic acid and carmine, saw in the subconjunctival areolar tissue peculiar rounded or oval bodies hanging on to the nerves "like a cherry on its stalk". He was apparently unaware of Krause's work: but Krause subsequently (1867) identified Lightbody's 'cherries' with his own *Endkolben*.

In the following year (1868) Rouget, using tissues macerated in dilute acid, found 'small, rounded bodies' situated at the tips of myelinated fibres in the human conjunctiva. He noted, as others had done, that these bodies were encircled by the myelinated pre-terminal portion of the nerve fibre, sometimes with a single turn, sometimes with more. Rouget disagreed with his predecessors on the subject of the connective tissue capsule, of which he saw no trace. The nuclei surrounding the central mass he confidently described as belonging to Schwann cells, and the 'capsule' itself as an expansion of the Schwann sheath; while the central mass appeared to be nothing other than a swollen blob of axonal substance. He noted, as did most subsequent authors, that the distribution of these bodies was very irregular; in one sector of an eye they were abundant, in another completely absent.

In 1870 Helfreich published a book on the nerves of the conjunctiva and sclera, in which he confessed that he had been unable to find end-bulbs in any of the animals he had investigated, with the exception of one unconvincing appearance in a frog. The only nerve endings he could see were fine unmyelinated fibres terminating close beneath the epithelium. Morano (1871) similarly described free nerve endings but was silent on the subject of end-bulbs. He was accused of piracy by Helfreich, and an undignified quarrel ensued.

In 1873 Ciaccio published a long and detailed article on the histology of the human conjunctiva. His account of the conjunctival nerves is based on pieces of tissue stretched out and gently scraped in order to remove the epithelium, and thereafter treated with either gold chloride or osmium tetroxide. He described a deep conjunctival plexus, which sends branches either to the cornea or to the conjunctival epithelium, where the fibres 'become pale', or end in 'Krause end-bulbs'. The latter are of various sizes, round or oval in shape, and of irregular distribution; in some areas there are none, in others many; in general, they are most abundant in the upper and outer quadrant of the bulbar conjunctiva. They consist of an outer investment of connective tissue, continuous with the sheaths of the nerve fibres supplying them, and an inner granular mass in which the 'pale' (i.e. non-myelinated) segments of the fibres terminate. These are served by one or more nerve fibres; most commonly there are two, deriving from a single fasciculus or as branches from a single stem fibre. In the calf, the fibre merely loses its myelin sheath and runs a straight course as a pale fibre in the centre of the bulb; in man, the myelinated segment forms a more or less intricate tangle outside the bulb (thereby obscuring its contents) before becoming pale and piercing it. The author was in no doubt as to the existence and the reality of these organs, and referred to Arnold's opinions with contempt. He also described a peculiar unencapsulated bush-like terminal expansion, which he encountered in a single preparation and to which he applied the term *fiocchetto*.

In the following year Waldeyer (1874) again cast doubt on the existence of end-bulbs in the eye. After a painstaking search he had been unable to find any, in

either man or calf. He drew attention to the striking difference between the two morphological types of end-bulb which previous workers had claimed to find in these two species, and to the fact that Krause and others had failed to find them in many other mammals. In Waldeyer's view, the descriptions and illustrations given of the end-bulbs in man imposed a severe strain on histological credulity.

In 1875, however, Longworth, working in Waldeyer's laboratory, succeeded not only in displaying end-bulbs, conforming to previous descriptions, in the human and bovine conjunctiva, but also in convincing Waldeyer, who thereupon published a recantation of his previous view as a tailpiece to Longworth's paper. Longworth noted, once again, that not every eye examined contained end-bulbs, and that when they were present their distribution was very irregular. He considered that the 'human' type of end-bulb was related to the Meissner corpuscle and the 'bovine' type to the Pacinian corpuscle.

In the same year Poncet (1875) published a review of the literature and the results of his own findings, which closely supported those of Ciaccio. His drawings, from whole-thickness preparations of conjunctiva stained with osmium tetroxide, tally in detail with Ciaccio's from similarly mounted gold-impregnated specimens.

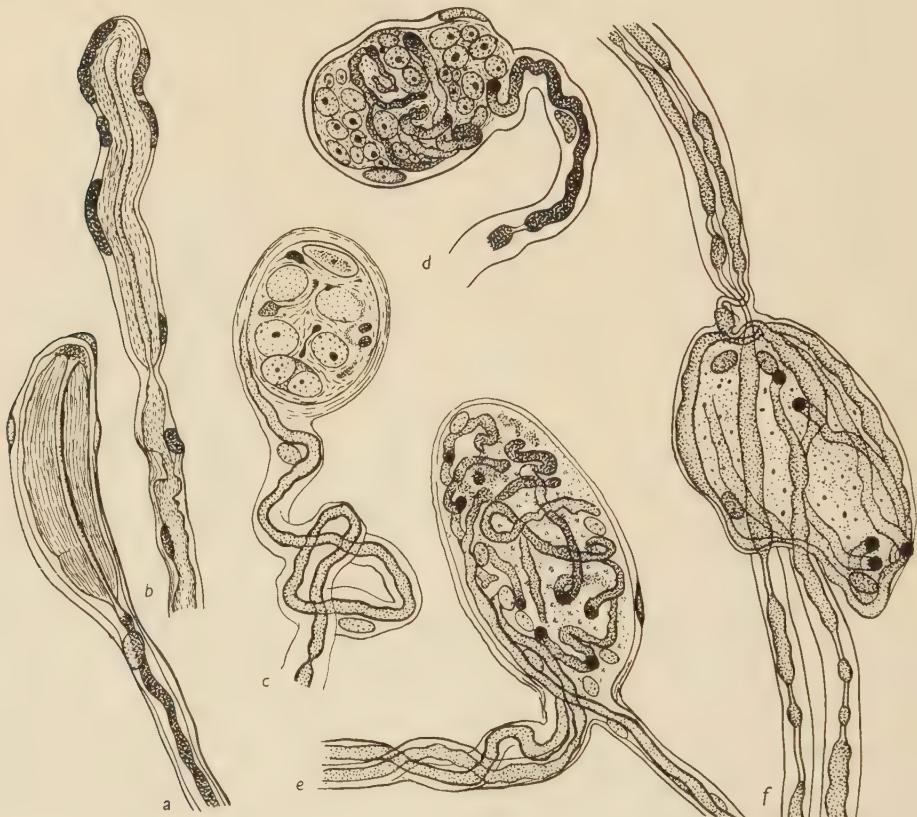
In 1876 Key & Retzius also reviewed the literature and gave a detailed, comprehensive and beautifully illustrated account of their own observations relating both to human and bovine material. Using tissues macerated in dilute acid as well as gold chloride and osmium tetroxide preparations, they described their findings in relation to what others had previously seen and described, with especial emphasis on the fact that they encountered great variations in form, amounting to a continuous series from the simplest club-like endings to large and complex end-formations, some of which appeared to consist of two or more end-bulbs stuck together (Text-fig. 2a-f). Like Poncet, they mention but do not explain the failure of some other workers to display end-bulbs.

In 1881 Krause himself published a general account of compact endings, with fresh illustrations which bear surprisingly little resemblance to what had now become the accepted picture of *Endkolben*. One sees what is presumably a myelinated fibre stopping abruptly at the edge of a solid ball of connective tissue cells; there is no suggestion of intricate tangles outside the 'capsule' or of fine ramifications inside. The text does not help to explain this discrepancy.

In 1884 Suchard gave a description, with illustrations, of 'human' and 'bovine' end-bulbs, confirming the observations of Ciaccio, Poncet and Longworth. His main concern was to show 'affinities' between the 'human' type and the Meissner corpuscle on the one hand, and between the 'bovine' type and the Pacinian corpuscle on the other.

A new era came with the introduction of methylene-blue staining. Dogiel published his findings in the eyeball as a whole in 1891. His general pictures of the conjunctival nerve plexus correspond well with those obtained by other methods; at various points, however, nerve fibres, after leaving the bundles, become varicose and enter into bizarre figures and convolutions (described as *Endknäuel* by the author) from which they emerge and run an uncomplicated course for a short way, only to enter into still other contorted figures (Text-fig. 3a). One fibre is depicted as passing through several such tangles, and eventually returning to and fusing

with the parent stem, close to the point where it originated. In his description, he points out that this serial arrangement is the normal pattern; if a fibre seems to terminate in a tangle, this is due to incomplete staining. In some of his preparations, Dogiel used a counterstain to show up the connective tissue elements, and in many cases was able to see a lightly staining 'capsule' surrounding an *Endknäuel*. This led him to identify these structures with Krause's *Endkolben*, though a comparison of his pictures with those of previous authors makes it difficult to see how such dissimilar objects could be identified. Incidentally, Dogiel claimed to have seen similar structures in the outer 2 mm. of the corneal margin, and to be the first to describe them.



Text-fig. 2. Redrawn from Key & Retzius (1876). End-bulbs from conjunctiva of ox (a) and (b) (cf. Pl. 1, figs. 6, 7; Pl. 2, fig. 16), and from man (c to f) (cf. Pl. 3, figs. 31, 32). Osmium tetroxide.

In addition to the pictures of encapsulated and unencapsulated tangles, Dogiel's paper gives a picture of an encapsulated structure which is strikingly different both from the traditional conception of an end-bulb and from the rest of his figures (Text-fig. 3b). Here there is no emergent fibre; a single fibre loses its myelin sheath and enters a connective tissue capsule, within which an axon of smooth contour but with fusiform swellings on its course branches and weaves in a complex but orderly

manner. This single drawing has since become a standard for depicting the morphology of 'Krause end-bulbs', and reproductions of it appear in many text-books.

In 1903 there appeared a detailed and comprehensive paper on the innervation of the human conjunctiva by Crevatin. He used both gold chloride and methylene-blue techniques on human material, and his illustrations seem to be from preparations of both kinds. His description of the 'typical' end-bulb tallies with those of Ciaccio and others: in addition, he was able to observe, in the interior of the bulb, a closely woven entanglement of fine, branching, naked nerve filaments, which was only partially obscured by the outer, myelinated entanglement (Text-fig. 4a-c). His illustrations show a wide variety of forms, including a number of compound

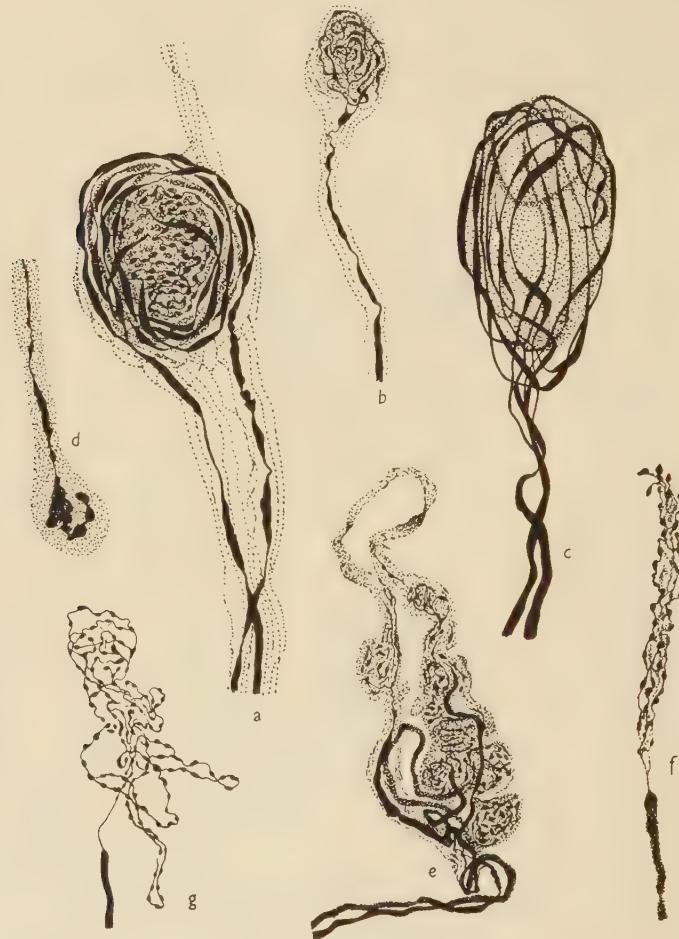


Text-fig. 3. Redrawn from Dogiel (1891). (a) Serially innervated *Endknäue* and (b) encapsulated ending from human conjunctiva. (b) is commonly reproduced as type specimen for Krause end-bulbs. Methylene blue.

structures, consisting of 'nests' of two or three, more or less 'typical' end-bulbs (Text-fig. 4e). Crevatin also described various other forms of nerve ending, distinguished from end-bulbs principally in the fact of possessing no capsule. They included *striscette*, knobbed, club-like endings of coarse nerve fibres; *fiochetti*, elongated, tangled skeins of varicose nerve fibres, occurring either in isolation or in association with a group of end-bulbs (Text-fig. 4f); and *intrecci*, *plessicini* and *reticelle*, loose, irregularly-shaped types of nerve entanglement, bearing a general resemblance to the *Endknäuel* depicted by Dogiel (Text-fig. 4g).

After Crevatin, there seems to have been little original work in this field until 1922, when Knüsel & Vonwiller described the microscopic and slit-lamp appearances

of the living human eye, subjected to vital staining. For demonstrating nerve elements, they used instillations of 1% aqueous methylene blue (without local anaesthetic) into the conjunctival sac. In this procedure, the first elements to appear were the most superficial nerve fibres, and end-bulbs, which could be seen hanging, like fruit from the twigs of a tree, at the ends of many of these fibres. The



Text-fig. 4. Redrawn from Crevatin (1903). (a) to (c) End-bulbs from human conjunctivae, showing outer, myelinated and inner, non-myelinated entanglements (cf. Pl. 2, figs. 18-23). (d) Simplest form of end-bulb (cf. Pl. 3, fig. 29). (e) Complex neural formation including several end-bulbs (cf. Pl. 3, figs. 25, 26). (f) Fiocchetto (cf. Pl. 1, fig. 4). (g) Reticella (cf. Pl. 1, fig. 3). Gold chloride and methylene blue.

size and distribution of the end-bulbs were variable. In general, they measured 40-50 μ in diameter, but in one subject they attained sizes of 80-200 μ . They were most frequent near the limbus, and more so in the upper sectors than in the lower ones. The largest number seen in a single eye was about 50. The appearance of the end-bulbs changed progressively as the dye took effect. At the stage of optimal staining, a fine nervous entanglement could be made out in the interior of the end-

bulb. Occasionally, nerve fibres could be seen connecting one end-bulb with another, in the manner described by Dogiel.

These authors also made the experiment of stimulating end-bulbs with *von Frey* hairs under vision through a corneal microscope, both during and after the process of staining. No definite sensations of touch or pain were evoked; nor, it is presumed, of cold, although the point is not specifically mentioned.

A similar technique of vital staining was used by Strughold & Karbe (1925) who sought to demonstrate a correlation between 'cold spots' and end-bulbs. They confined their investigation to Dr Strughold's right eye, in which, during the course of three experiments, they found only five compact endings, three of which formed a serially innervated group.

In 1934 Egorow, using methylene blue in the manner of Dogiel, published an account of nerve endings in the guinea-pig eye. Her illustrations show a variety of more or less complex nerve terminals, including a large encapsulated tangle of fine, branching filaments, which was seen near the corneo-scleral margin.

It seems that little use has been made of silver impregnation for the demonstration of conjunctival end-bulbs. The only histological account based on silver preparations that we have come across is that of Boeke (1932). This author is not concerned with the differentiation of 'species' of compact endings—indeed, he shows the modern tendency to put Krause end-bulbs, Ruffini endings, corpuscles of Golgi-Mazzoni and the rest into a single group, and displays some impatience with the long differential descriptions of earlier writers. He is interested rather in finding a common structural principle in these endings. From his description and illustrations of end-bulbs from the human conjunctiva, it would seem that the tangle of terminal branches of the entering fibre lies free within a protoplasmic syncytium, containing a variable number of nuclei, which constitutes the 'core' of the structure: outside this core is a nucleated capsule, which is continuous with the endoneurium of the entering axon. No mention is made of an outer myelinated entanglement. The more complex of the two end-bulbs which he depicts bears a close resemblance to Dogiel's much-reproduced figure.

In summary, during the fifty years following Krause's original demonstration of compact nerve endings in the conjunctiva, eighteen workers published their findings on this subject. Among these there was general agreement on the existence of diffusely arranged free nerve-endings but some sharp disagreements on the existence of compact endings in the conjunctivae of men and oxen. Seven of those who reported their presence remarked that their distribution was always highly irregular, and that in some cases they could not be found at all. Six authors extended their observations to other mammals, but for the most part, their findings were negative. Only Krause claimed to have found compact endings in the conjunctivae of a wide range of animals.

Regarding the morphology of the compact endings, different authors give strikingly different accounts. Two authors, however, describe a wide variety of observed forms, from which it may be conjectured either that they had examined a larger number of specimens, or that they had examined their specimens more thoroughly than the others. It is unfortunate that not a single author actually states the number of specimens examined.

During the last fifty years, little more has been added to our knowledge of the innervation of the conjunctiva, but the subject has acquired theoretical importance from the work of von Frey and his collaborators, who have claimed to show that the compact endings of the conjunctiva (and similar endings elsewhere in the body) are specific receptors for the sensation of cold.

MATERIAL AND METHODS

(1) Material for histological examination

This was taken from 74 mammalian eyes. Table 1 indicates the distribution of the specimens among the animals examined as well as the manner in which the specimens were prepared for microscopical examination. The figures refer to the number of eyes in which the nerve elements were successfully stained or impregnated throughout large areas of bulbar conjunctiva. The eyes from laboratory animals were obtained before or shortly after death from an overdose of Nembutal. The eyes of sheep and oxen were taken within an hour of slaughtering. Human material was taken from cadavers, 6-48 hr. after death.

Table 1. Material examined

Animal	(1) Whole mounts of bulbar conjunctiva dissected from eyeball				(2) Sections cut from eyeballs with bulbar conjunctiva <i>in situ</i>				Total
	Methylene blue	Osmium	Silver	Gold	Methylene blue	Osmium	Silver	Total	
Albino mouse	2	2	
Albino rat	6	.	3	.	.	.	1	10	
Rabbit	6	.	1	.	.	1	2	10	
Cat	1	.	1	.	1	1	2	6	
Sheep	.	.	1	.	.	.	1	2	
Ox	4	2	4	.	.	1	4	15	
Monkey	1	1	2	1	2	1	2	10	
Man	1	.	8	.	.	1	9	19	
Total	21	3	20	1	3	5	21	74	

The specimens for microscopical examination took the form either of sections cut from the fixed, undissected eyeball on the freezing microtome, or of whole mounts of pieces of dissected conjunctiva. Sections were cut in a variety of planes, including the vertical and tangential. Whole mounts were obtained as follows; after fixation, the bulbar conjunctiva was gently stripped from the sclera in five or six sectors, each attached to a sector of cornea. The pieces were pinned out with the epithelium downwards, and the loose areolar tissue, which contains the main nerve bundles but not the conjunctival nerve plexus, was carefully snipped away with fine scissors. Much depends on this procedure, for the areolar connective tissue fibres attract an irregular deposition of metallic silver, which may completely obscure the nerves running between them and the epithelium.

In heavily pigmented specimens, as for instance those from monkeys and oxen, the epithelium was gently scraped away from the conjunctival connective tissue. Artefacts, due to the recoil of nerve fibres stretched to breaking point, are sometimes observed as the result of these procedures. Such artefacts are usually easy to recognize.

In the case of albino rats and mice, it was found possible to prepare the anterior halves of the eviscerated eyes as whole mounts, for the tissues are thin enough to allow translucent specimens to be obtained even following silver impregnation. A number of radial incisions enabled flattening to be achieved without notable distortion of nerve fibres.

(a) *Methylene blue*

(2) *Histological methods*

For methylene blue staining, a spreading factor, 0·025% hyaluronidase (Hyalase, Benger) was first injected beneath the conjunctiva. This was followed by a subconjunctival injection of 0·02–0·05% methylene blue (Merck) in physiological saline. Whenever possible these were done in the living, anaesthetized animal; good results were obtained, however, in animals which had been recently killed, and in one case in a man who had died 6 hr. previously. After 15–30 min. staining, the anterior half of the eyeball was immersed in ice-cold 7% ammonium molybdate for 12–16 hr. To obtain whole mounts, the conjunctiva was removed from the sclera, rapidly dehydrated in several changes of redistilled absolute alcohol, and cleared. To obtain sections, the specimens were removed from the ammonium molybdate, washed and placed in 10% formalin neutralized with calcium carbonate, where they remained for 1 or 2 days before sections were cut on the freezing microtome. The sections were then washed, and rapidly dehydrated.

The advantage of methylene blue staining is that in zones where the concentration of dye is optimal it gives a very clear and undistorted picture of nerve fibres of all calibres. However, it is very difficult to obtain, consistently, specimens which are evenly stained over a wide area, for conditions leading to optimal staining are very critical. Overstaining, whether from excessive concentration of dye or from too prolonged application, leads to gross distortion and even disruption of nerve fibres as well as to non-specific staining, such elements as blood vessels, lymphatics and reticular and other connective tissue fibres readily taking up the dye. Local patches of understaining, some of which are probably due either to uneven spreading of the dye or to decolorization during processing, are unfortunately common.

(b) *Osmium tetroxide*

Myelinated sheaths were stained by first injecting a 1/3% solution of osmium tetroxide subconjunctivally into an enucleated eye and then immersing the whole eyeball in a solution of the same concentration for 12 hr.

(c) *Gold chloride*

Gold chloride impregnation after the manner of Ranvier proved to be an unsatisfactory method in our hands. Usually, the capillary network showed up with extreme clarity, but the nerve fibres never had the sharpness of outline seen in good methylene blue or silver preparations.

(d) *Silver*

In the case of human eyes, we had to rely chiefly on silver impregnation. For this, a method was needed which gave consistent results and was specific enough to enable us to examine thick preparations satisfactorily. In the first place, various

modifications of the Bielschowsky technique were tried, but in the end we adopted a technique developed in our laboratory by Schofield (1957). This gave the most consistent and rewarding results and enabled us to examine whole mounts satisfactorily. Axis cylinders of all calibres are specifically impregnated, and appear black against a light brown background, which yields sufficient details of the tissue elements present to render counter-staining unnecessary. There is little, if any, distortion of the axons and little haphazard precipitation of metallic silver on other than neural elements. This point is of considerable importance in view of the possible existence of pathological changes in the nerve fibres of the specimens at our disposal.

In only a few cases can we claim to have made a complete examination of the whole of the bulbar conjunctiva in any given eye. A certain amount of material is invariably wasted in specimens obtained by cutting frozen sections, and complete and uniform staining throughout the tissue by methylene blue is rare. In whole mounts impregnated with silver, there are nearly always a few patches where the tissue is not translucent, owing to incomplete removal of areolar tissue or slight folding. We can claim, however, that in all of the 74 eyes listed above, we were able to examine a reasonably large area of the bulbar conjunctiva, at least in its circum-corneal region.

(3) *Vital staining with methylene blue*

(a) *The material* consisted of volunteers, five male and three female, between the ages of 9 and 49 years. On these, a total of fourteen experiments was carried out.

(b) *Staining technique.* The eye was first bathed for about 30 sec. in an eyebath with a 2% solution of cocaine hydrochloride containing 1 part in 2000 of adrenaline hydrochloride. Bathing was repeated at about 5 min. intervals, until the conjunctiva was anaesthetic to light touch and completely blanched. This took about 10 min. The eye was next bathed with a solution of 1% methylene blue (Hoechst, Medicinal) in distilled water diluted with 4% cocaine hydrochloride to give a final concentration of dye of between 0.3 and 0.5%. This was also repeated two or three times during the next half an hour.

Thereafter, the progress of the staining could be followed by means of a hand-lens. For closer inspection, the adjustable brow-pad and chin rest of a slit-lamp microscope were used to support the head. The stained conjunctiva was examined through a microscope fitted with lenses having focal lengths of 3.0 and 4.5 cm. respectively, and an eye-piece magnifying ten times. Photographs were taken by means of a 35 mm. photomicrographic camera attachment in the eye-piece position and a ring-shaped flash tube fitted around and a little behind the microscope lens.

This method proved to be an invaluable complement to the histological techniques described above. Although the magnification which could be usefully employed was too small for the finest structural details to be observed, it gave an over-all view of the pattern of innervation of an intact conjunctiva, of a kind well-nigh impossible with histological methods. It also provided a check on our histological material, in that the eyes examined were those of healthy subjects, not of hospital patients in the course of a disease. Most important, it enabled us to observe changes in the innervation of an individual eye at varying intervals of time.

Regarding the completeness of staining of nervous elements by this method, we regard it as comparable with the best silver or methylene blue preparations of whole mounts. Thus, in every case (except one, in which the inner half of the conjunctiva remained unstained) we were able to see, in all parts of the bulbar conjunctiva, the details of the conjunctival nerve plexus precisely as we had seen it in histological specimens. In some respects, in particular in evenness of staining and in the absence of non-specific staining of connective tissue fibres, the vital method showed considerable advantages over the conventional histological methods. In many cases, it was noticed that the staining of the conjunctiva did not progress simultaneously in all areas; thus, different areas might reach the stage of optimal staining at different times. This effect is presumably due, among other things, to local differences in the permeability of the tissue, and one may infer that the same factor may be responsible for the uneven staining so often observed in methylene blue preparations of detached pieces of tissue. Here, again, the vital method shows up to advantage, in that all areas pass at some period through a stage of optimal staining.

The one case in which staining was clearly incomplete was that of a child of twelve. A second attempt was made some months later on the same eye. On this occasion, no staining whatever occurred—not even of epithelial cells, which are normally the first to take up the stain. We were unable to account for this phenomenon.

In view of some earlier accounts of the intense discomfort caused by methylene blue staining, it may seem strange that we used children as subjects. In fact, it was soon found that the procedures described caused no severe pain. The treated eye usually begins to ache within 2 hr. of the application of the dye; but this aching is completely stopped in a matter of seconds by bathing with cocaine. Repeated applications of cocaine are required during the next 4 hr. or so, after which the aching disappears. On the following day, the conjunctiva is a little hyperaemic but not painful. It is usually anaesthetic to light touch, and remains so for 2 or 3 days, after which sensibility returns to 'normal' (i.e. light touch feels the same as it does in the control eye). No untoward after-effects have been observed.

The prolonged period of anaesthesia is probably to be ascribed to the action of the dye on the nerves, since it is well known that the effect of cocaine wears off in a few hours.

OBSERVATIONS

A. *Histological*

(1) *Laboratory animals*

The first specimens which we examined were of fresh material from young healthy animals kept under artificial conditions in a laboratory animal house. They came from mice, rats, rabbits, cats, macaques and baboons. These showed nerve bundles emerging from the sclera at various points, and running, in company with major vessels, in the subconjunctival connective tissue, where they formed a characteristic plexus, with many radially-directed small bundles of fibres coursing towards the limbus. The more anterior bundles crossed the limbus to form a plexiform network in the more superficial layers of the cornea. Offsets from the conjunctival plexus passed into smaller nerve bundles which they eventually left as individual axons of various calibres. These, after branching extensively, became

thinner, lost first their medullary sheaths, then their (visible) Schwann sheaths and finally tapered to invisibility in relation either to the walls of blood vessels or to the conjunctival epithelium (Pl. 1, fig. 2). As our primary concern was with the presence or absence of end-bulbs, we have not attempted a detailed or quantitative analysis of the pattern and distribution of the nerve fibres ending freely in the conjunctiva. From what we have seen, however, we have found no occasion to disagree with the qualitative descriptions given by Ciaccio and others on this subject.

Exceptions to this mode of termination by branching and tapering to invisibility were found in only five instances in thirty-eight specimens examined. In one specimen, a methylene-blue preparation from an apparently healthy rat, a terminal axon formation was seen (Pl. 1, fig. 3), which closely resembled what Crevatin described as a *reticella* (cf. Text-fig. 4g). This, as can be seen, consisted of an irregular entanglement of branched, varicose fibres, with no sign of encapsulation. In the second specimen, a gold chloride preparation of a macaque conjunctiva, there were two comparable, though somewhat elongated, formations of branched, varicose fibres, again without capsules (Pl. 1, fig. 4). These appeared to us to correspond to Crevatin's *fiochetti* (cf. Text-fig. 4f). The third specimen, a silver preparation from a cat, showed two small 'end-bulbs' similar to, but smaller than, those said to be classical for the ox (Pl. 1, fig. 5, and compare Text-figs. 1b and 2a). As the innervation of the remainder of these eyes conformed to the diffuse pattern described above, we felt compelled to regard these five instances of compact endings as exceptions to the normal rule. We were encouraged in this view by the fact that several previous workers had been unable to find end-bulbs in the conjunctiva of animals other than ox and man. The possibility that they were technical artefacts will be discussed later.

(2) Slaughterhouse material

Two eyes from sheep, and fifteen from young oxen, were examined. All the animals were slaughtered less than an hour before the specimens were collected. The eyes were either placed in fixative or treated with methylene blue on the spot. In these, a precisely similar pattern of innervation was seen to that found in laboratory animals, namely a plexiform network of fibres giving rise to individual axons (Pl. 1, fig. 1), terminating diffusely in relation either to blood vessels or to cells of the epithelium.

No compact or encapsulated endings were found in either of the sheep's eyes, or in those of the first eight bovine eyes examined, all of which were from young calves. However, on examining material from a batch of eyes from 2-to 3-year-old bullocks after silver impregnation, we at last had some confirmation of what had been described in the older literature. In randomly scattered regions, unusually thick myelinated nerve fibres of irregular contour running just beneath the epithelium ended in characteristic sausage-shaped 'organs' (Pl. 1, figs. 6, 7; cf. Text-figs. 1b and 2a, b). On the outside of each there was a 'capsule', which was seen to be a continuation of the endoneurial sheath of the fibre supplying it. Underlying this were elongated and somewhat flattened nuclei, indistinguishable, apart from the flattening, from those of Schwann cells in the neighbourhood. Within this outer layer was a structureless granular substance; and in the centre, the densely staining

club-like termination of the axon. There was generally a marked constriction of the axon at the point of entry, where the myelin sheath ended abruptly. Outside the 'organ', connective-tissue cells sometimes showed a slight degree of concentric orientation, giving the impression of a passive thrusting aside of connective-tissue elements by an expanding body.

This 'classical' form of bovine 'end-bulb' was not the only type observed. In some cases, a number of short thick branches took the place of the single central axonic club (Pl. 1, figs. 8, 9); in others, one or two fine fibres, arising from the club-like axonic swellings, could be seen running in the interior of the 'bulb' (Pl. 1, fig. 10); and in one specimen two 'bulbs' were seen in which fine fibres sprouted from the sides or tip of a terminal club and formed a more or less spirally-wound skein inside the 'bulb' (Pl. 1, fig. 11). There were also 'bulbs' in which the terminal club was clearly defined, but the 'bulb' as a whole was smaller and the surrounding sheath and its nuclei were only faintly discernible.

In some instances, large irregular myelinated nerve fibres which terminated in typical bovine 'end-bulbs' gave off collaterals which behaved quite differently. In one case the collateral arose from a nodal point, but rapidly changed direction and pursued an irregular course towards the epithelium. Shortly before reaching it, it lost its myelin sheath, arborized into numerous branches which, in turn, lost their Schwann sheaths and finally terminated diffusely (apparently by attenuation) in relation to the cells in the epithelium. In other words, one and the same axon gave rise to both a compact and a diffuse terminal. The axis cylinders forming such collaterals usually contained fusiform swellings (of the kind frequently seen in regenerating nerves) which were largest at points where the axon changed direction abruptly. An example of an arrangement of this kind is given in Pl. 2, fig. 16. In this instance, the axis cylinder, derived from the collateral having the largest number of swellings, ended in a small conical mass reminiscent of a growth cone (Pl. 2, fig. 16, arrow on montage).

The dimensions of the bulbs were in agreement with previous descriptions—namely, 65–140 μ in length by 25–35 μ in breath. In the matter of distribution, our findings also agreed with those of most previous authors. In a single eye, there were areas where 'bulbs' occurred with a density of 4 per sq.mm. and other areas, of a square centimetre or so, where there were none. In some eyes, as already mentioned, we found none at all; in others, only one or two.

Two additional features were observed which we believe to be significant. In the first place, the nerve-fibres supplying the 'bulbs' were without exception 'abnormal' in appearance. In some cases, the visible abnormality consisted merely in varicose irregularities in the axon. These were either smooth in outline and fusiform in shape or irregular in both shape and outline (Pl. 2, figs. 12, 13; compare Text-fig. 4*a*, *b* and *d*). In others, the impregnation of the axon was discontinuous, and the myelin sheath, which is not usually impregnated at all, was diffusely impregnated and very irregular in calibre (Pl. 2, fig. 14). This feature was very conspicuous in the specimen which provided the biggest yield of 'end-bulbs'. Here, of course, the suspicion of technical artefacts arises; we concluded, however, that there was more to it than this, since not only were there normal-looking fibres of even calibre in the same preparation, but in individual nerve bundles there were both smooth and irregularly

varicose fibres running side by side with normal-looking fibres of coarse and fine calibres; and wherever an abnormal fibre could be traced to its termination, it was found to end in a 'bulb' (Pl. 1, figs. 6, 7; Pl. 2, figs. 12-14).

The second observation was that, in at least three cases, the 'bulb' was clearly intra-neural, that is, included along with a number of passing nerve fibres within the perineurium of a nerve bundle, which was locally distended to accommodate the 'bulb' (Pl. 2, fig. 15).

In short, we have found 'end-bulbs' of the bovine type in the bulbar conjunctiva of five young bullocks' eyes, locally abundant in some cases, sparse in others. We failed to find them in two young bullocks' eyes and eight young calves' eyes. In general, they conform to previous descriptions; but to these we would add our observations of more complex forms, the occurrence of intra-neural 'bulbs' and the abnormal appearance of the nerve fibres serving them. These observations are summarized in Table 2.

(3) *Human material*

We were unable to obtain perfectly fresh material. Six eyes were obtained from cadavers, between 6 and 48 hr. after death. We were also supplied with a number of formalin-fixed eyes, nine from diabetics, and four from non-diabetic patients. In most cases fixation had begun 36-48 hr. after death. The subjects were mostly elderly or middle-aged.

It was found that late fixation did not prevent the successful silver impregnation of nerve axons. Even when the epithelium itself showed post-mortem changes such as exaggerated intercellular spaces, the axons were generally almost normal in appearance, being of relatively even calibre over long distances. They were arranged in a precisely similar pattern to that found in the other eyes which we had examined, namely a plexiform network ending diffusely in relation either to blood vessels or to epithelial cells. Thick fibres of markedly uneven calibre, when they could be followed to their termination, were nearly always found to enter into some peculiar type of end-formation. In our single osmium tetroxide preparation (from a 12 hr. cadaver), the myelin sheaths showed irregularities of outline, which from their appearance may well have been due to post-mortem changes.

No characteristic differences were observed between the eyes from diabetics and the others, except that in four of the 'diabetic' eyes there were places where the nerves emerging from the sclera, instead of running in simply-branched bundles, formed loose and much less formal entanglements (Pl. 2, fig. 17). This feature was not observed in any 'non-diabetic' eyes; but the material was too limited to enable one to judge whether these entanglements were in any way attributable to the particular disease process from which the patients were suffering.

In two of the eyes examined, no compact nerve-endings were seen. In two, single solitary compact endings were found: in four, a solitary group of three or four such endings: in others, there were several such groups, while in four specimens compact endings were locally abundant—up to three or four per low-power field—though even in these, large areas were apparently devoid of them. On account of the technical difficulties mentioned earlier, precise figures of the distribution of these endings in different eyes cannot be given; it became obvious, however, that great

variations occur as between one eye and another, and between different areas of the same conjunctiva. There was no correlation between numbers of complex endings and the ages of the subjects, all of whom had died from disease after the age of 30. Lacking material from young and healthy persons, we did not consider it profitable to seek for correlations with the patients' medical histories. It may be mentioned here that compact endings were never seen in any part of the cornea. These observations are also summarized in Table 2.

The variety of the forms assumed by these endings was very striking. It has been mentioned that Crevatin described, illustrated and classified a wide variety of forms. We have seen endings which fall neatly into each of his pigeon-holes; but we have also seen enough intermediate forms to convince us that Crevatin's classification is an artificial one. In general, we can say that the specimens in which compact endings are most abundant show the widest scatter of forms, while in those which show only a few, these tend to be similar in form, and of a comparatively simple type.

Thick, irregular fibres ending in knobbed or thorny 'clubs', corresponding to Crevatin's *striscette*, have been seen, though it is often difficult to be sure that these constitute the true terminations of fibres. These 'clubs' may possess a few short twigs sprouting near their tips (Pl. 3, fig. 29; compare Text-fig. 4d). Such forms merge into what Crevatin would have described as a simple 'end-bulb'—a cluster of short twigs forming a knot less than $20\ \mu$ in diameter. Pl. 2, fig. 18, is an example of a simple 'end-bulb' which has an additional point of interest; the axon from which it springs has fusiform swellings along its course, and gives rise to a collateral which pursues a complicated course towards the surface, where it ends in a diffuse arborization (cf. Pl. 2, fig. 16, and Text-fig. 4b, d). From such an 'end-bulb' there is a continuous series of endings in which fibres are more branched, and the branches perform more complicated contortions (Pl. 2, figs. 19, 20). In 'end-bulbs', which may be $80\ \mu$ or more in diameter, the thick parent fibre or fibres (there may be two or more) are often seen to wrap themselves round the 'bulb'; their main branches, still thick and presumably myelinated, form an investing entanglement either in the periphery of the 'bulb', or at or near its hilum; and the fine terminal branches are seen weaving intricate patterns in the interior (Pl. 2, figs. 21–23). This last type clearly corresponds with the forms described by the earlier workers (compare Text-figs. 2c, d; 3b; 4a–c). In a few cases we have seen compound 'end-bulbs' in which up to four 'bulbs' of the more elaborate type lie closely packed together (Pl. 3, figs. 24–26). These too were described by Crevatin (Text-fig. 4e).

An equally natural series appears to link the simple 'end-bulb' with the elongated forms described by Crevatin (which, incidentally, would probably be classified by some workers as 'Ruffini endings'). These are sometimes isolated, sometimes closely associated, at their hila, with 'end-bulbs' from which they seem to differ in shape and size alone. Similarly, there does not appear to be a sharp distinction between the 'end-bulb' with its densely woven fibres and globular shape, and the looser, irregularly shaped entanglements called *reticelle*, *intrecci*, or *plessicini* by Crevatin (Pl. 3, figs. 27, 28; cf. Text-fig. 4b, g).

On one point we are unable to follow Crevatin, and that is the question of

encapsulation. According to him, there is an investment of one or two layers of connective tissue surrounding each 'end-bulb', but no such investment around any of the other types of ending. In our material we have seen no clear evidence of non-nervous tissues entering intimately into the structure of 'end-bulbs'. As with the bovine material, Schwann nuclei are generally numerous in the periphery of the 'bulb', and sometimes occur inside it; in the larger specimens, there may be some roughly concentric arrangement of connective-tissue cells which give the appearance of having been thrust aside by an expanding body; but this apparent 'capsule' is more often than not deficient on one side. The point is of some theoretical importance, and will be discussed later,

Another point of difference from Crevatin is that, whereas he was of the opinion that fine terminal nerve twigs (even those served by axons of even calibre) are almost always coarsely beaded, they do not appear so in our silver preparations. In discussing this point Crevatin concluded that the beads were 'natural' since they were seen equally in gold chloride and methylene-blue preparations. We are inclined to regard beading of this kind as a technical artefact, for both the nerves and other tissue elements are less distorted (i.e. correspond more closely to those in the fresh cornea under phase-contrast conditions) in specimens impregnated by the silver technique we are using than is customary in specimens prepared by other silver methods which, in turn, are far less distorted than those impregnated with gold or stained with methylene blue. We ourselves have regularly encountered beading of the finest terminal nerve twigs but only in gold chloride and methylene-blue stained material (see Pl. 1, figs. 3, 4; and compare Pl. 1, fig. 2, and Pl. 3, fig. 28).

It was observed that every compact ending was served by at least one thick, myelinated fibre, usually of uneven calibre; often, however, there were fine, even-calibred fibres running in association with these and apparently joining in the terminal entanglement. The degree of varicosity of the thick fibres seemed to be generally related to the complexity of the ending; thus, the fibres serving the simpler endings were often even in calibre over a long stretch, more or less normal in appearance but of unusually large diameter. The varicosities referred to are either fusiform swellings, reminiscent of those seen along the course of regenerated axons where they mark the sites of temporary obstruction (Weddell, 1942), or irregularly shaped swellings resembling those seen either in the early stages of nerve degeneration or following prolonged compression of nerve bundles (Denny-Brown & Brenner, 1944*a, b*). It is worth mentioning here that varicose fibres of both kinds, though not mentioned in the literature, are clearly delineated in many of the illustrations, including those of Dogiel and Crevatin (see Text-figs. 3*b* and 4*a*).

We were unable to answer the question, first raised by Dogiel, whether some or all 'end-bulbs' possess both entrant and emergent nerve fibres. Most commonly, one or more fibres enter (or leave) the 'bulb' at one place, which one may term the 'hilum'; sometimes fibres are seen leaving (or entering) in another part of the 'bulb'; but whether these are independent fibres, or the continuation of one of the others, cannot be determined owing to the complexity of the entanglement within the bulb. In one or two specimens, however, we have seen a fibre forming either a simple unbranched entanglement or a more complex branched entanglement at some point in its course, thereafter continuing as a single axon for a short distance

before terminating in an 'end-bulb'. These entanglements would correspond to what Krause called *Knäuel*. Pl. 3, fig. 30, is an example of such a formation. The axon 'a' enters the entanglement 'b', where it gives off a number of abbreviated offshoots which end in small club-like processes. The stem axon itself proceeds to the end of the entanglement where it divides into two. Both daughter axons then pursue a recurrent course and emerge from the entanglement at 'c'. Here they fuse into a single axon which after a short straight course terminates by branching in a complex manner and forming a large 'end-bulb' (cf. Text-figs. 2e, f, and 4a).

In addition to silver impregnation, we made spreads of a human conjunctiva stained with methylene blue, and sections of an eyeball fixed and stained with osmium tetroxide. The former showed no compact endings. In the latter, we found a group of three in a single section, which closely resembled forms described by the earlier workers (Pl. 3, figs. 31, 32; cf. Text-fig. 2d, e). In these, the myelin of the axons and of their branches ramifying on the outside of the 'bulb' could be clearly seen. A feature of this, which can be seen in several of the illustrations of Key and Retzius and others, is that the tangled nerve fibres are only intermittently myelinated—that is, a myelinated segment often comes between two non-myelinated segments (Text-fig. 2f). The arrow in Pl. 3, fig. 31, points to a non-myelinated segment of an axon encircling one of the 'bulbs' in our preparation. The irregular contours of the myelin sheaths may well be a post-mortem change.

In summary, we have seen, in material from cadavers, compact nerve endings of a wide variety of types, most of which have been described at one time or another in the past. We were able to confirm previous observations on the extremely irregular distribution of these endings. We have the impression that the fibres ending in this manner are in some way 'abnormal'; that the terminations themselves consist of nervous and Schwann elements only; and that their 'capsules', when they are present, are merely prolongations of the normal coverings of the nerve fibres entering into their formation. Finally, in Table 2, we have summarized all our histological observations.

Table 2. *Summary of histological observations*

Source	Eyes		
	No. examined	No. with compact endings	No. of compact endings seen
Laboratory animals	38	3	5
Slaughterhouse material	17	5	1 eye: more than 20 2 eyes: 5–20 per eye 2 eyes: 1–2 per eye
Human autopsies	19	17	4 eyes: more than 20 per eye 7 eyes: 5–20 per eye 4 eyes: 3–4 per eye 2 eyes: 1 per eye

B. *Observations in vivo*

The first elements to take up the blue stain are randomly scattered superficial cells of the epithelium. Fortunately, the colour fades during the course of the experiment, so that when nerve staining is most complete the stained epithelial cells have largely become invisible again. Next, after an interval varying from

20 min. in some subjects to over an hour in others, isolated segments of the conjunctival nerves are seen as blue threads. At about the same time, compact endings, if they are present, begin to show themselves; however, they cannot be identified with certainty until their parent axons take up the dye selectively. More and more 'normal' axons are seen, including the deeper-lying bundles and the finer superficial branches, which appear to end by attenuation, until after a period which varies between $\frac{3}{4}$ and $2\frac{1}{2}$ hr., the whole conjunctival nerve plexus is stained. As has been mentioned before, it often happens that staining in one sector lags behind that in another. The nerves of the cornea are also stained, but the nerve bundles entering it appear to be cut short at the limbus unless slit lamp conditions are substituted for direct illumination. In one case, a few capillaries containing blue-stained erythrocytes were seen in the conjunctiva; apart from these, and the epithelial cells mentioned above, there was no specific staining of non-nervous structures. Pl. 4, figs. 33 and 34, give a general impression of the pattern of the conjunctival vessels and nerves in two young subjects in zones free from large axons and 'end-bulbs' after staining *in vivo*.

As regards compact endings, Table 3 gives a summary of our findings. It will be seen that they reinforce the histological observations in several respects, particularly in showing the gross disparity in numbers as between different subjects (over 50 in one subject, none at all in another) and between different sectors of the same eye (for example, 27 in one quadrant, 6 in another, and none in the remaining half). As a general rule, we were able to confirm the observation of Ciaccio and others, that compact endings tend to occur most frequently in the upper and outer quadrant of the conjunctiva. In the matter of shape and size, too, gross differences were observed. In the case of very small (under 20 μ) endings, it was sometimes difficult to decide whether they were in fact nerve endings or epithelial cells which had not become completely decolorized; and it is possible that some of our counts were too low, owing to our having ignored some of the smallest and simplest end-formations. Admitting this, we were left in no doubt whatever as to the extreme variability of compact endings in the matter of size, form and distribution. The fact that the compact endings regularly showed a strong affinity for the dye, by staining earlier and more densely than the neighbouring nerve fibres, renders it very improbable that the observed irregularity in their numbers was due to either uneven or incomplete staining.

Another point in confirmation of the histological findings was the almost invariable association of compact endings with axons of large diameter and irregular contour. Not every ending was served by an obviously abnormal fibre, although minor degrees of abnormality would not be visible under the magnifications available; and, in one case, in an eye which was being stained for the second time, some fibres having an exceptionally large diameter were seen which, towards their terminations, became suddenly very fine and apparently ended by attenuation. Apart from this, the association of fibres of exceptionally large diameter and compact endings was too close to be fortuitous.

In every case of repeated staining, there was a striking diminution in the number both of compact endings and of exceptionally large nerve fibres. The larger endings had disappeared completely, and only a sprinkling of smaller compact terminals was

Table 3

Subject	Sex	Age	Eye	Date	Findings
AGMW	M.	49	L	20. iii. 57	40 compact endings counted, of very varied shapes and sizes, irregularly grouped in all quadrants, but most plentiful in the upper outer quadrant. Many abnormally thick axons served the 'end-bulbs' but many normal axons seen, apparently ending by attenuation.
DRO	M.	42	L, 1st expt.	11. vi. 56	50 compact endings counted, of very varied shapes and sizes, irregularly grouped in all quadrants but most plentiful in the upper outer quadrant. Many abnormally thick axons served the 'end-bulbs' but many normal axons were seen apparently ending by attenuation.
DRO	M.	42	L, 2nd expt.	3. vii. 56	10 compact endings counted, irregularly scattered in the outer quadrants. All the 'end-bulbs' were small and rounded in shape. No abnormally large axons served them nor were any seen elsewhere in the bulbar conjunctiva. A full complement of normal axons was seen apparently ending by attenuation throughout the conjunctiva.
DRO	M.	42	L, 3rd expt.	21. ix. 56	5 compact endings counted, randomly scattered in the upper quadrants. 4 of the 'end-bulbs' were small and 1 of them was large. 3 abnormally large axons served the 'end-bulbs'; there was a full complement of normal axons apparently ending by attenuation throughout the bulbar conjunctiva.
DRO	M.	42	R, 1st expt.	24. x. 57	40 compact endings counted, of very varied shapes and sizes (although 16 of them were exceptionally large and arose from abnormally thick axons.) The 'end-bulbs' were irregularly grouped in all quadrants but most plentiful in the upper outer quadrant. There was a full complement of normal axons throughout the bulbar conjunctiva.
DRO	M.	42	R, 2nd expt.	30. i. 57	4 small rounded compact endings counted in the upper outer quadrant. 5 abnormally thick axons were seen, 3 of which appeared to end by attenuation. The other 2 served 'end-bulbs'. Some capillaries filled with immobile blue-stained red cells were seen in the early stages of this experiment.
PPL	M.	28	L	25. x. 56	33 compact endings counted, of varied shapes and sizes. 27 were seen in the upper outer quadrant and 6 in the lower outer quadrant; none were seen in the inner quadrants. Some abnormally large axons served them. There was a full complement of normal axons apparently ending by attenuation.

Table 3 (*continued*)

Subject	Sex	Age	Eye	Date	Findings
BP	F.	23	L	5. vii. 56	7 compact endings of varied shapes and sizes arose from a single axon of abnormally large diameter in the upper outer quadrant. No 'end-bulbs' or abnormally large axons were seen elsewhere in the conjunctiva. There was a full complement of normal axons which apparently ended by attenuation.
BP	F.	23	R	28. viii. 56	3 compact endings counted in a single group in the upper outer quadrant. No 'end-bulbs' were seen elsewhere; nor were any abnormally large axons encountered. There was a full complement of normal axons which apparently ended by attenuation.
JD	F.	18	L	13. iii. 57	11 small rounded compact endings counted. There were 10 in the upper outer quadrant and 1 in the upper inner quadrant. No 'end-bulbs' were seen elsewhere; nor were any abnormally large axons encountered. There was a full complement of normal axons which apparently ended by attenuation.
CNO	F.	14	L	29. viii. 56	25 compact endings of varied shapes and sizes counted, arranged in 6 groups sited irregularly around the limbus. A single abnormally thick axon was seen; it appeared to terminate by fraying out into a tassel-like formation. There was a full complement of normal axons which appeared to end by attenuation.
RNO	M.	12	R	19. ix. 56	14 compact endings of varied shapes and sizes counted in the upper outer quadrant; none were seen elsewhere. No abnormally thick axons seen. Full complement of normal axons was seen in the outer quadrants. Staining of the inner quadrants was incomplete.
SJO	M.	9	R	20. ix. 56	0 compact endings counted in the bulbar conjunctiva. No abnormally thick axons were seen. There was a full complement of normal axons throughout the bulbar conjunctiva.

seen. There appeared to be no decrease in the density of the innervation of the conjunctiva in general. Thus, one must suppose either that the endings had become refractory to the stain, or that they had been destroyed by the dye. The latter alternative appears more plausible, for methylene blue is known to interfere with tissue respiration, and in general those structures which are most vulnerable from this point of view become blue first.

In summary, the following points emerge from our observations on vital staining with methylene blue:

(1) There are gross disparities in numbers of compact endings as between individuals and as between different sectors of a given eye. When present, they tend to be commonest in the outer and upper quadrants. Too few subjects have been examined to make a clear correlation between age and numbers of compact endings; however, it is worth remarking that the largest number was seen in the oldest subjects, and the smallest number in the youngest.

(2) Compact endings are usually, though not invariably, associated with thick nerve fibres having an uneven calibre.

(3) When the same eye is stained for a second time, there is a striking diminution in the number of compact endings although no obvious decrease in the local density of innervation.

Pl. 4, figs. 35–41, illustrate some of the compact endings which we saw in the stained human eye.

Pl. 4, fig. 39, shows a single compact ending seen in the upper outer quadrant of the bulbar conjunctiva of the right eye in a male aged 42 years. The axon serving it is large, irregular in outline and pursues a tortuous course; the ‘normal’ axons in the nerve bundles on the left of the picture are in sharp contrast. More than forty compact endings, of which sixteen were $100\ \mu$ or more in diameter, were seen in this eye. Under low magnification they looked very like those in Pl. 4, fig. 40, which is of a small area of bulbar conjunctiva in the upper outer quadrant of the left eye in a male aged 49 years. More than forty compact endings were counted in this eye also. The photograph (Pl. 4, fig. 40) was taken soon after staining; thus some of the epithelial cells were still dark blue and many ‘normal’ nerves had not yet taken up the dye.

Pl. 4, fig. 35, shows one of four small compact endings seen in the upper outer quadrant of the same eye as that from which Pl. 4, fig. 39, was taken, but after an interval of 14 weeks had elapsed from the time the eye was first stained. In addition to the four compact endings a full complement of ‘normal’ nerves was seen which apparently terminated by attenuation in the usual way. In other words, the ‘abnormal’ axons, together with all but four of the ‘end-bulbs’ they served, had disappeared in the interval between the experiments. Another point to be noted is that the terminal shown is small and served by a fine axon, which is presumably non-myelinated, for all that can be seen is a pathway of Schwann cells whose nuclei can be seen at regular intervals along its course. This is what was once known as a Schwann ‘band’. In methylene-blue preparations of skin examined with the light microscope, Schwann ‘bands’ are *only* seen when fine regenerating axons have either reached them or are within a few microns of them (Weddell, 1942).

DISCUSSION

The two most striking features of the earlier work on the innervation of the conjunctiva are: first, the contradictory findings of different authors; and secondly, the extreme variability of form and distribution of compact nerve endings reported by many of them. It seems highly probable that the first was the direct result of the second—in fact, that the contradictions arose from limited observations on material which was liable to great variations. Even so, this very variability, which is far in

excess of what is found in the innervation of other parts of the integument, and which is amply borne out by our own observations, is an interesting fact, which seems to call for some attempt at explanation. Hitherto, so far as we know, the attempt has not been made.

Regarding the biological significance of compact nerve-endings, it seems to have been assumed on all sides that they are of the nature of sensory end-organs, playing a specialized rôle in the sensibility of the integument. The assumption is a natural one, and in the case of hair follicles and the corpuscles of Meissner and Pacini, we have no desire to quarrel with it. The exact sensory function of these structures is still not altogether clear, but there is no question of their regular occurrence in characteristic zones, or of the relative stability of their numbers and their morphology.

The case is different with the compact nerve endings of the conjunctiva, and we now believe there is evidence to justify us in casting doubt on the view that they are 'sense organs' in the usual meaning of the term, and in proposing an alternative view.

It is well known from the work of Perroncito (1905), Cajal (1928) and others, that following experimental section of a peripheral nerve, a certain proportion of the fibres in the proximal stump are obstructed in their efforts to grow peripherally, as a result of the closure of the distal end of the so-called digestive chamber. The axonal stump may thicken and assume bizarre forms, and the digestive chamber may become filled with sprouting collaterals, with growth cones at their tips. These 'sterile' endings may assume a large size—up to $40\ \mu$ in diameter, according to Cajal—but sooner or later they degenerate and are absorbed into the surrounding tissue. There are, in addition, endings of similar appearance, which Cajal describes as 'semi-fecund'. In these, one or more collaterals are seen escaping from the digestive chamber into the surrounding tissue, and terminating some distance away in typical growth-cones.

A very characteristic form of 'sterile' ending is the apparatus of Perroncito. Here the digestive chamber forms a closed, elongated bag containing the thick axonal stump, and a number of fine, collateral fibres which pursue a spiral retrograde course, seeking, as it were, to escape by coursing round and round their prison wall (Text-fig. 5a). In a second type (Text-fig. 5b) no fine collaterals are seen, but the axonal stump is irregularly thickened and shows numerous short club-like excrescences.

Of a third type, the 'nervous spool', Cajal writes: "[It] is in reality nothing more than a persistent apparatus of Perroncito, whose central and spiral fibres have grown very much in thickness and in length, have acquired partially or completely a sheath of Schwann and a medullary sheath, and terminate either inside or outside the sheath of the old tube, in medium-sized or large capsulated balls" (Text-fig. 5c).

We have selected these three types of 'sterile' ending from the wide variety described by Cajal, because we have seen structures closely resembling each of them in our present material. Thus, the bovine 'end-bulb' shown in Pl. 1, fig. 11, shows a structure strongly reminiscent of the Perroncito spiral (Text-fig. 5a); those of Pl. 1, figs. 8 and 9, are very similar to the clubbed endings of Text-fig. 5b; while the 'nervous spool' (Text-fig. 5c) has obvious features in common with the typical human 'end-bulb' (Pl. 2, fig. 20; Pl. 3, fig. 27).

It was by structural similarities of this nature that we were led, in the first place, to consider the possibility of an alternative view of conjunctival 'end-bulbs'—namely, that they might represent stages in a continuous or intermittent process of growth, decay and regrowth in the distal ends of peripheral nerve-fibres.

Taken alone, of course, such structural resemblances prove nothing (we would point out, incidentally, that the assumption which we are calling in question—namely, that 'end-bulbs' are sense organs—was itself based on no more than a real or fancied structural resemblance between 'Krause end-bulbs' on the one hand and the corpuscles of Meissner and Pacini on the other). There are, however, certain additional points of evidence which strongly suggest that the similarity between 'end-bulbs' and the 'sterile' or 'semi-fecund' spools described by Cajal is more than superficial.



Text-fig. 5. Redrawn from Cajal (1928). (a) Apparatus of Perroncito (cf. Pl. 1, fig. 11). (b) Tuberose end-formation (cf. Pl. 1, figs. 8–10). (c) Nervous spool (cf. Pl. 3, fig. 27). Silver impregnations.

In the first place, there is the appearance of the axons supplying a large proportion of compact endings. The thickening and irregularity, which is seen both in silver preparations and in vitally stained human material, must be taken as *prima facie* evidence of a disturbed physiological state. In addition, the 'human' type of compact ending shows a remarkable feature in that the myelin sheath of the terminal portion of axon, and of the outer entanglement of thick fibres, is frequently discontinuous, so that the fibres exhibit alternate myelinated and unmyelinated segments. This feature is clearly brought out in Key and Retzius's

illustrations (Text-fig. 2*f*), and we have observed it in our material (Pl. 3, fig. 31, at arrow head). It is probably of some significance, in view of the fact that intermittent myelination is a well-recognized feature of growing nerve fibres (see Speidel, 1932, 1933, 1935).

The second observation is the finding of 'bovine' endings embedded in the substance of a nerve bundle (see Pl. 2, fig. 15). Their presence here is rather difficult to explain on the old assumption that they are sense organs. This difficulty does not arise if they are regarded as mere by-products of some process of neural growth.

It will be appreciated that the theory we are putting forward would remove the 'Krause end-bulb' and other compact endings in the conjunctiva from the category of sensory end-organs which includes the corpuscles of Meissner and Pacini. We believe, in any case, that there are good morphological grounds for doing so. In the case of Meissner's and Pacini's corpuscles there is a definite framework, composed of non-nervous elements, among which the sensory nerve terminals insinuate themselves (Cauna, 1956, 1958). Thus, it is legitimate to speak of a Pacinian corpuscle as an organ, which is innervated. In the 'end-bulb', on the other hand, we have so far failed to detect any elements other than axis cylinders, myelin and Schwann sheaths and the terminal expansion of the endoneurium which constitutes the capsule of the 'end-bulb'. In other words, the innervation constitutes the whole 'organ'. A reservation must be made in the case of the bovine 'end-bulb', which contains a granular, apparently acellular core surrounding the central axonal stump. The nature of this material is unknown. If our theory is correct, it is probably the cytoplasm of enlarged Schwann cells containing the 'digested' débris of an earlier Perroncito formation.

Two problems arising out of the work of Dogiel have received little attention from subsequent histologists. These consist in his finding, as a regular occurrence in the conjunctiva and elsewhere, of serially innervated *Knäuel* (see pp. 325 and 326) and of branched fibres, one branch of which terminates in a *Knäuel* and the other as a diffuse arborization. If the *Knäuel* in question are to be regarded as specialized nerve endings, Dogiel's findings can hardly fail to be puzzling, as they would involve a radical revision of our conception of the anatomy of peripheral nerve endings. We ourselves reject the easy explanation which regards these particular formations described by Dogiel as artefacts resulting from mechanical distortion, partly because it is difficult to envisage how myelinated nerve fibres of the size depicted could be locally twisted into such bizarre shapes in the course of preparation for microscopical examination, and partly because we ourselves have seen neural formations of a similar kind which we have good reason to believe are not artefacts. For one thing, the entanglements have certain morphological characteristics which would not be seen in artificially coiled and twisted nerves (see pp. 338 and 339 and Pl. 3, fig. 30). These appearances, we repeat, are hard to explain if the 'end-bulbs' in question are sense-organs. If they are the result of 'obstruction' in the course of regeneration, the problem disappears. It will be remembered that Cajal described, in the proximal stump of a divided nerve, a type of 'semi-fecund' ending in which a collateral (rarely two) succeeded in penetrating the capsule of the nervous spool, and ran a further straight and direct course, to end as a typical growth cone in the scar. There is no difficulty, then, in supposing that in the conjunctiva such

an emergent collateral might either form a normal diffuse arborization and terminate in the epithelium, or meet a further obstruction and produce another 'sterile' or 'semi-fecund' ending (see pp. 335 and 337, and Pl. 2, figs. 16, 18).

A question, which has so far not been satisfactorily answered, arises in relation to the marked structural difference between the typical 'bovine' and the typical 'human' type of ending. It has been suggested by several investigators that the former is a modified Pacinian, and the latter a modified Meissner corpuscle. There are obvious difficulties in this view. On the other hand, if one is prepared to abandon the assumption that they are specific 'end-organs' and consider their possible analogy with 'nervous spools' and other formations described by Cajal, one can see how both types might develop from an initial state corresponding to the spiral of Perroncito. In the case of the 'bovine' type, the spiral fibres would wither away, leaving either a simple or a branched axonal stump in the centre; while in the 'human' type, the spiral fibres would persist, or even multiply, while the preterminal portion of axon, restricted by the endoneurium in its efforts to increase in length, would lay down successive coils in the outer layer of the 'spool'. As to why the one process should be more favoured in the ox and the other in man, we can offer no suggestion; but we find it easier to believe that certain tissue differences between man and ox result in somewhat different expressions of a similar growth process, than that the conjunctivae in the two species are equipped with different types of sense-organ.

One would also like to know why men and oxen, as compared with common laboratory animals, should be especially favoured with 'end-bulbs'. The only observation we can make on this point is that, of the species we have examined, only man, and to a lesser extent the ox, expose a large area of bulbar conjunctiva to the outside world. In all the rest, the limbus is almost completely concealed by the eyelids. Other mammals will have to be examined and experimental investigations carried out before this question can be satisfactorily answered.

There remains the question why there should be such wide variations in the number of compact endings in the conjunctivae of different members of one species, and in different regions of one conjunctiva. We do not know the answer; on the other hand, we regard this variability as one of the most significant pieces of evidence that the endings in question are to be regarded as stages in a process, rather than as specialized sense-organs. One does not find a similarly haphazard distribution in the taste-buds of the tongue, the hairs of the skin, or the tactile corpuscles of the finger tips. The most natural supposition is that the structures in question are labile (our experiments with repeated methylene-blue staining have shown this to be so) and may come and go in response to changes in either the physical environment or in the *milieu interne*.

The conception of lability in the peripheral nervous system is not new. Speidel (1940-1) has made direct observations of alternate progressive and regressive changes occurring in the nerves of the tail of the tadpole. Zander & Weddell (1951) showed that after a partial keratotomy nerve fibres spread into the denervated area from the neighbouring segments of cornea, only to regress as the normal innervation became re-established. Weiss (1941) has generalized these and similar observations into a concept of a dynamic process by means of which the density of innervation

of any given area remains approximately constant. This concept is probably applicable particularly to the system of diffuse (free) endings which is found over the whole integument; and it involves the notion of a continuously alternating process of advance and withdrawal of the tips of the terminal arborizations of this system. In the present context, we are making the additional suggestion that in certain mucosal surfaces, including the conjunctiva, it may happen from time to time that a fibre is obstructed in its distal growth, and responds by the formation of 'sterile' or 'semi-fecund' terminals.

Mention has already been made of the experiments of Strughold & Karbe. In these, Dr Strughold's right eye was first explored for 'cold spots' and subsequently subjected to vital staining on three separate occasions. In the result, fifty-two 'cold spots' were identified, evenly distributed over the bulbar conjunctiva, and recorded on a chart. Vital staining revealed a total of five compact endings, three of which formed a compact group. This group, and the other two endings, were each found to lie within a 'cold spot'. These findings were held to confirm von Frey's view that the 'Krause end-bulb' was the specific receptor for sensations of cold. It is difficult to see how these experiments can be regarded as evidence for such a theory. It would seem, on the contrary, that only one of two conclusions could be drawn from them—either that the staining technique was inadequate to display the fifty-odd 'end-bulbs' postulated by the theory, or that sensations of cold can be evoked without the mediation of 'end-bulbs'. The second alternative has in fact been shown (Lele & Weddell, 1956; Sinclair, Weddell & Zander, 1952) to hold good in the case of the human cornea and hairy skin, and in experiments (at present incomplete) in the case of the conjunctiva.

Thus, although many questions remain to be answered, we feel that we are in a position to give a general answer to the questions which we originally proposed.

First, as to the occurrence of compact nerve endings in the mammalian conjunctiva, it seems that such endings are absent or rare in some animals, and relatively common in others. Among the latter, there are gross variations between one individual and another, and between different areas of a single conjunctiva.

Secondly, these endings do not conform to any constant morphological type. The 'end-bulbs' described by Krause are merely two forms selected from a wide variety of compact endings.

Thirdly, the assumption which has prevailed hitherto, that these endings possess some specific sensory function, must be regarded as unproven. It follows that experimental work based on this assumption must be regarded with suspicion.

SUMMARY

1. The literature on compact sensory nerve endings in the conjunctiva is reviewed.
2. Histological examination of the conjunctivae of various mammals, and vital staining of the human conjunctiva with methylene blue, lead to the following conclusions:
 - (a) The great majority of the nerve fibres entering the conjunctiva end as diffuse arborizations (free nerve endings) in relation to blood-vessels or to the epithelium of the conjunctiva and cornea.

- (b) In the conjunctivae of common laboratory animals compact nerve endings are rare.
- (c) Compact endings of many types, which include the 'end-bulbs' described by Krause, occur in very variable numbers in the conjunctivae of men and to a lesser extent oxen.
- (d) When present, they are very irregularly distributed.
- (e) Various lines of evidence suggest that these endings represent stages in cycle of growth and decay in certain peripheral nerve fibres rather than specialized 'sense-organs'.
- (f) Various problems arising out of the older literature are considered in the light of this theory.

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EXPLANATION OF PLATES

PLATE 1. Figs. 1 and 2 from frozen sections, remainder from whole mounts. Fig. 3 is from methylene blue, fig. 4 from gold chloride preparation. Remaining figures from silver preparations.

Fig. 1. Offsets from conjunctival plexus passing to epithelium. Ox.

Fig. 2. Fine nerve filaments ending by attenuation in conjunctival epithelium. Macaque.

Fig. 3. Loose neural tangle. Note coarse beading of fibres in this and following figure. Rat. (Cf. Text-fig. 4g.)

Fig. 4. Elongated formation of varicose nerve fibres. Macaque. Whole mount. (Cf. Text-fig. 4f.)

Fig. 5. Two sausage-shaped 'end-bulbs'. Cat.

Figs. 6, 7. Large sausage-shaped 'end-bulbs', with simple club-like central axons. Ox.

Figs. 8, 9. 'End-bulbs' with branched central axons. Ox. (Cf. Text-fig. 5b.)

Fig. 10. Club-like axonal swellings giving rise to fine fibres inside 'end-bulb'. Ox. (Cf. Text-fig. 5c.)

Fig. 11. Fine collateral fibres running spiral course inside 'end-bulb'. Ox. (Cf. Text-fig. 5a.)

PLATE 2. All figures from silver preparations. Figs. 20 and 23 from frozen sections, rest from whole mounts.

Figs. 12, 13. Normal and thick varicose nerve fibres lying side by side in small bundles. The varicose fibres terminated in 'end-bulbs'. Ox.

Fig. 14. Impregnation of myelin sheath with defective impregnation of axis cylinder. Arrows point to nodes of Ranvier. Vertical fibre terminated in 'end-bulb'. Ox.

Fig. 15. Intra-neural 'end-bulb'. Ox.

Fig. 16. Enlargement of 'end-bulb' in Pl. 1, fig. 7. Montage shows collateral emerging proximal to 'end-bulb' and terminating diffusely. Arrow indicates 'growth-cone'. Ox.

Fig. 17. Loose entanglement of axons in conjunctival nerve plexus. Diabetic patient.

Fig. 18. Simple 'end-bulb'. Note collateral fibre. This seen to end diffusely in epithelium. Human.

Figs. 19–23. 'End-bulbs' of increasing complexity. Human.

PLATE 3. Figs. 31 and 32 from whole mounts stained with osmium tetroxide, rest from silver impregnated whole mounts.

Figs. 24–26. Compound 'end-bulbs'. Human. (Cf. Text-fig. 4e.)

Figs. 27–29. Various 'atypical' end formations. Human.

Fig. 30. Complex entanglement (*Knäuel*) arising on course of nerve fibre which terminated in 'end-bulb'. *a* axon proceeding to entanglement, *b* entanglement, axon gives rise to abbreviated offshoots which end in club-like processes then divides into two; *c* two emergent axons fusing, single axon proceeds on straight course for short distance before terminating in 'end-bulb'. Human.

Figs. 31, 32. 'End-bulbs' with myelinated fibres ramifying around central core. Arrow on Fig. 31 is pointing at non-myelinated segment. Human. (Cf. Text-figs. 1a and 2c–f.)

PLATE 4. All figures flash photographs of conjunctivae of living persons, stained with methylene blue.

Fig. 33. Lower inner quadrant bulbar conjunctiva; corneal margin on right. Nerve bundles radially arranged; axons have a regular contour and pursue a direct course. Neither large axons of irregular contour nor 'end-bulbs' are seen. Female, aged 18 years.

Fig. 34. Upper outer quadrant bulbar conjunctiva; axons arising from conjunctival plexus and serving diffuse terminals. Neither coarse axons of irregular contour nor 'end-bulbs' seen. Male, aged 9 years.

Fig. 35. Upper outer quadrant bulbar conjunctiva: one of only four 'end-bulbs' seen. Axon is certainly non-myelinated, the pathway in the picture is a series of Schwann cells and their nuclei (Schwann 'band'). 14 weeks previously this same eye was full of 'end-bulbs' (740). Male, aged 42 years. (Cf. fig. 39.)

Fig. 36. Inner quadrant bulbar conjunctiva at limbus; 'end-bulb' served by large coarse fibre of regular contour pursuing a straight course. Male, aged 14 years.

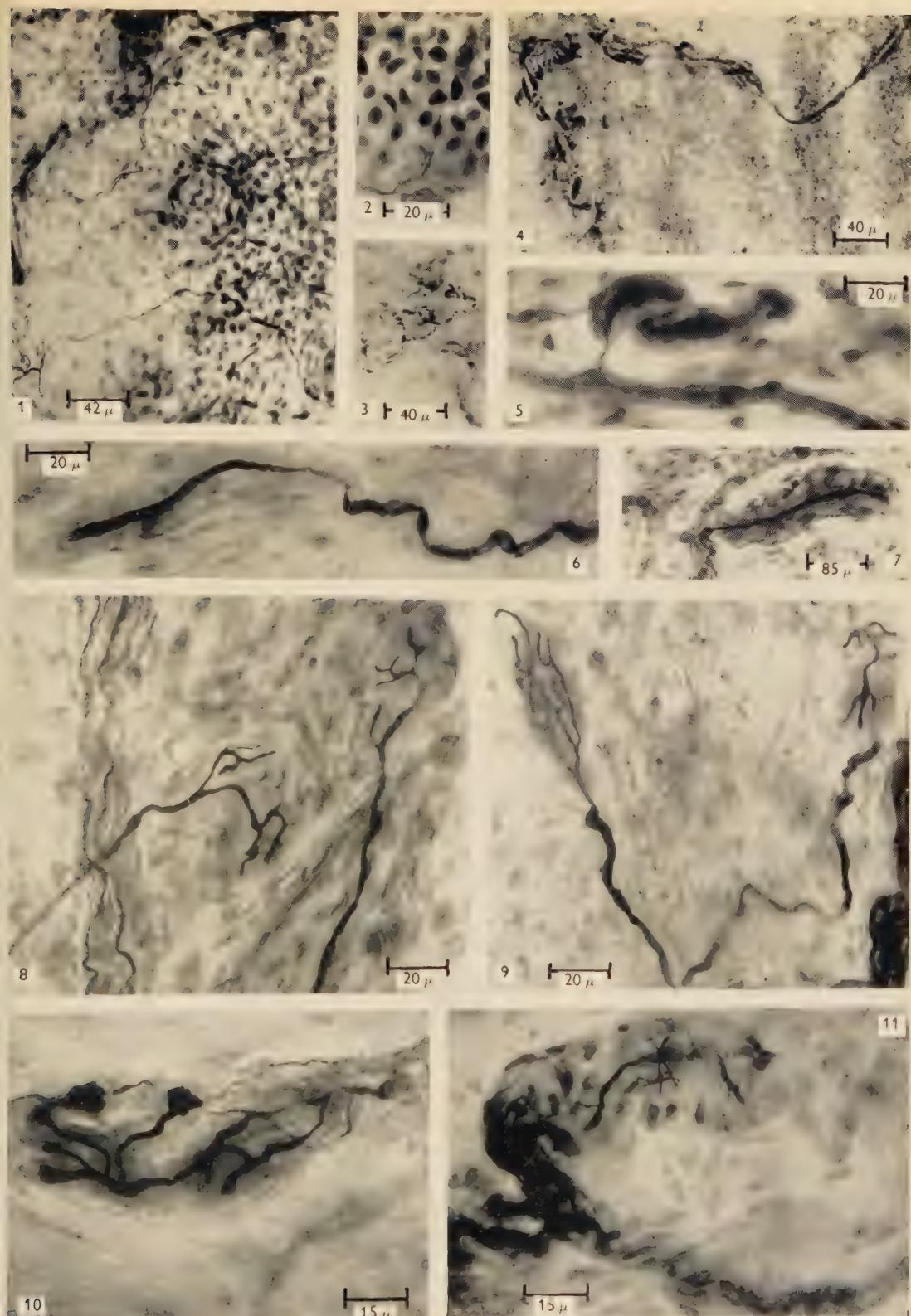
Fig. 37. Upper outer quadrant bulbar conjunctiva: one of 14 'end-bulbs'; it is small and deeply stained. The fibre serving it pursues an irregular course. Compare fig. 4 from same eye. Male, aged 12 years.

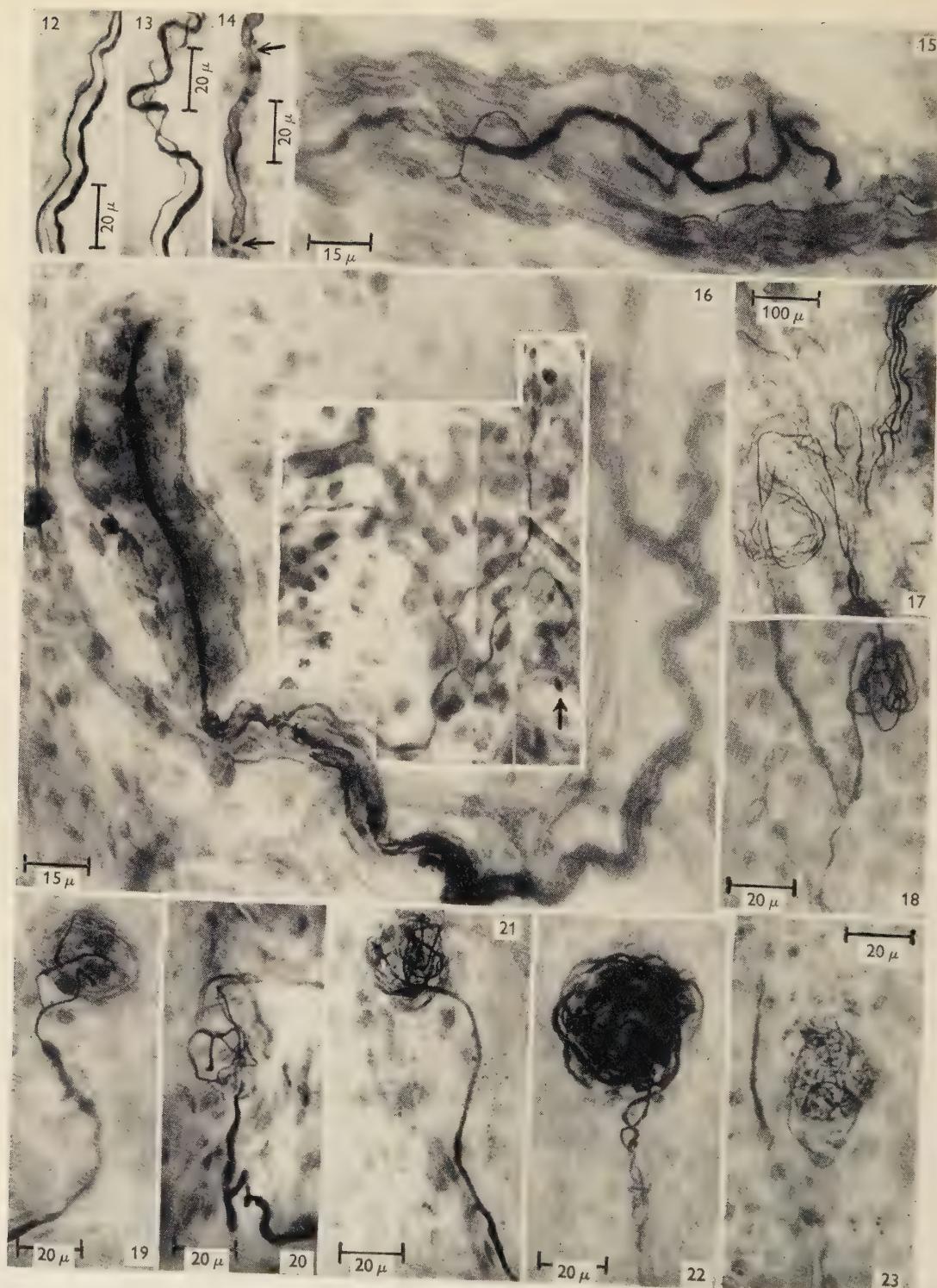
Fig. 38. Upper outer quadrant bulbar conjunctiva: clusters of 'end-bulbs', of various sizes and complexity, served by fibres of irregular outline pursuing a relatively straight course. Male, aged 12 years.

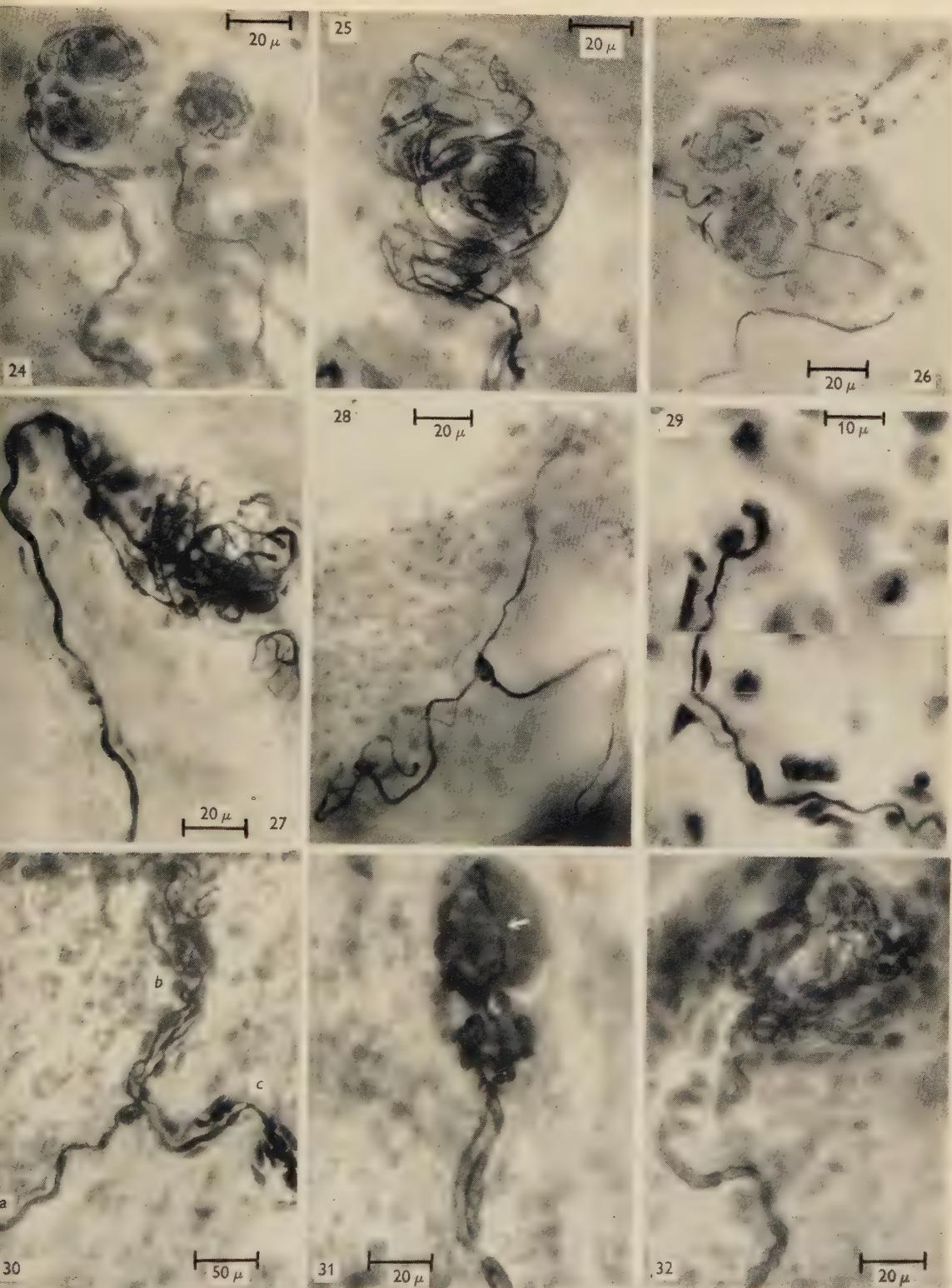
Fig. 39. Upper outer quadrant bulbar conjunctiva: single large 'end-bulb' served by a coarse fibre of irregular contour pursuing a very tortuous course. On the left there are bundles of normal nerve fibres; they are much finer, regular in contour and pursuing a straight course. More than 40 'end-bulbs' were counted in this eye: 14 weeks later, only 4 'end bulbs' were seen, but a full complement of 'normal' nerves. Male, aged 42 years. (Cf. fig. 35.)

Fig. 40. Upper outer quadrant bulbar conjunctiva: many 'end-bulbs' of different sizes served by large axons of irregular contour, pursuing tortuous courses. Picture taken shortly after staining, some epithelial cells are still dark blue. Male, aged 49 years.

Fig. 41. Upper outer quadrant bulbar conjunctiva: one of 14 'end-bulbs'. It is very large, and served by an axon of irregular contour pursuing a somewhat irregular course. Compare fig. 37, which shows another 'end-bulb' less than 2 mm. distant. Male, aged 12 years.







THE INTRINSIC NERVE CELLS OF THE CARDIAC atria of mammals and man

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INTRODUCTION

Davies, Francis & King (1952) made a detailed study of the intrinsic ganglia of the cardiac ventricles in a number of mammals and man, and the purpose of the present work is to extend the investigation to include the atria.

Among the earliest workers, Scarpa (1794) and Lee (1849 *a* and *b*) noted with the naked eye numerous nerves covering the whole surface of the heart of various mammals and man, and considered swellings on these nerves to be ganglia. Remak (1844), Lee (1849), and Kölliker (1865) maintained that the nerves pass into the depth of the myocardium and carry the nerve cells with them, though this was challenged by Cloetta (1853) and Vignal (1881). Cloetta, who was one of the earliest to study the heart microscopically, denied the existence of nerve cells in the heart. Since the latter part of the nineteenth century, a very extensive literature has developed concerning the sites and nature of the nerve cells in the atria, and this has been reviewed by S. Michailow (1912), Perman (1924), Francillon (1928) and Stöhr, Jun. (1932).

In general, it may be said that the commonest situation among various mammals and man, in which atrial nerve cells have been noted by numerous workers, is the wall of the right atrium in the region of the opening of the right pre-caval vein (superior vena cava). Other territories include the region of the opening of the post-caval vein (inferior vena cava), the atrioventricular junction, the inter-atrial groove, the region of the openings of the pulmonary veins (including the adjacent dorsal wall of the left atrium) and the atrial septum (among the muscle fibres). For the main part these nerve cells were found to be situated beneath the epicardium, though some authors have noted nerve cells among the myocardial fibres of the atrial walls (including the atrial septum). A few workers have described ganglia at and near the origins of the aorta and pulmonary artery, though in the present work these ganglia are regarded as belonging to the ventricular series. Special attention has been devoted by several observers to the relation of nerve cells to the atrial part of the cardiac 'conducting system', namely the sinu-atrial (s.a.) and atrioventricular (a.v.) nodes and the atrioventricular (a.v.) bundle. Many detailed studies have also been made on the finer structure and types of nerve cells in the atria and of their possible functional nature.

A survey of the previous work has revealed that there is much difference of opinion concerning both the situation and the types of atrial nerve cells, so much so as to warrant the present re-investigation.

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MATERIALS AND METHODS

The hearts studied were as follows:

Ornithodelphia. Platypus (*Ornithorhynchus*) (1), Echidna (*Echidna* sp.) (1).

Didelphia. Wallaroo (*Macropus robustus rubens*) (1).

Monodelphia. Insectivora: hedgehog (*Erinaceus europaeus*) (1), mole (*Talpa europaeus*) (1).

Rodentia. Rat (*Rattus*, laboratory white rat) (2), guinea-pig (*Cavia porcellus*) (2).

Lagomorpha. Rabbit (*Oryctolagus cuniculus*) (2).

Carnivora. Cat (*Felis*) (4), dog (*Canis familiaris*) (3).

Cetacea. Porpoise (*Phocaena communis*) (1).

Artiodactyla. Ruminants: domestic ox (*Bos taurus*) (3), sheep (*Ovis aries*) (1); non-ruminants: pig (*Sus scrofa*) (2).

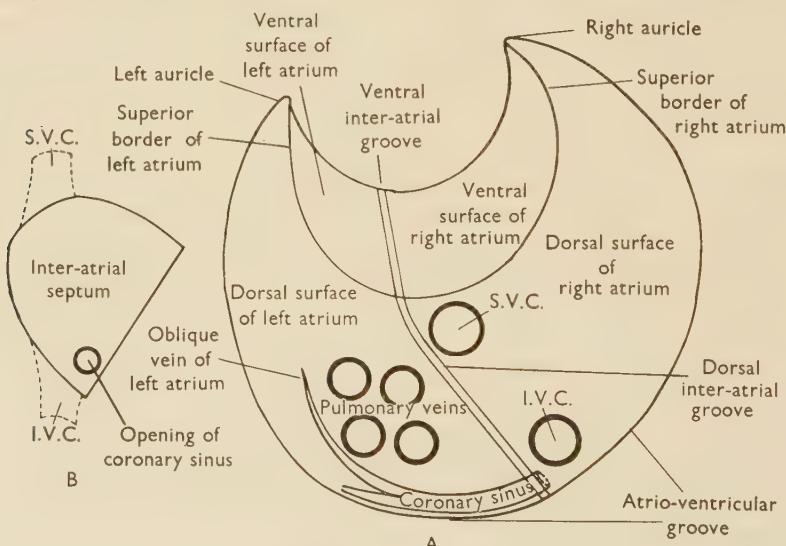
Perissodactyla. Horse (*Equus caballus*) (1).

Chiroptera. Fruit bat (*Pteropus* sp.) (1).

Menotyphla. Tree shrew (*Tupaia* sp.) (1).

Primates. Rhesus monkey (*Macacus rhesus*) (1), man (*Homo sapiens*) (3).

The numbers in parentheses indicate the numbers of specimens of each that were examined.



Text-fig. 1. Standardized diagram of epicardial surface of right and left atria and the inter-atrial septum.

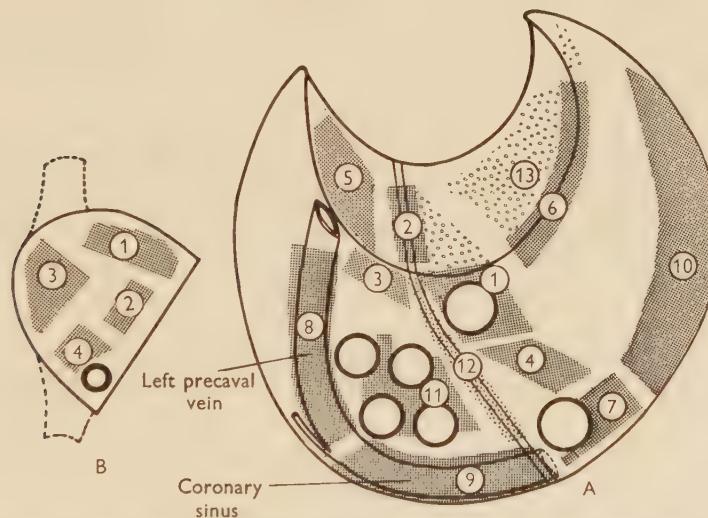
In most cases serial transverse paraffin sections of whole hearts were prepared; in others, particularly the larger hearts, several blocks of the atrial walls were removed and paraffin or frozen sections made of each. The various staining techniques employed included haematoxylin and eosin, iron haematoxylin and picrofuchsin (van Gieson), Masson's trichrome stain (using light green), pyridine-silver (Blair & Davies, 1935), and the Bielschowsky-Gros, Ranson and Smith Quigley silver methods. In the early stages of the work, graphic reconstructions from projected drawings

were made to indicate the position of the nerve cells, but this proved to be exceptionally time-consuming.

Two standardized diagrams (Text-fig. 1 A, B) were therefore devised, and on them the positions of the ganglia in the various hearts were plotted as accurately as possible in their relation to well-defined cardiac structures. The first (Text-fig. 1A) represents the atria viewed from their cranial aspect so that the ventral and dorsal surfaces are visible. The second (Text-fig. 1B) represents the atrial septum, viewed from the right side. In some of the hearts a left pre-caval vein was present (instead of the oblique vein of the left atrium); this is depicted in Text-fig. 2, which also indicates the general disposition of the ganglion fields that were located microscopically and will be described subsequently.

The number of sections used for the reconstructions varied from every 5th in the smaller hearts to every 50th or 60th in the larger ones, the number being indicated in the Text-figures by a fraction (e.g. the fraction 1/10 in the platypus heart in Text-fig. 3).

From the totality of observations made, the following numbered ganglion fields (Text-fig. 2) were delimited, many of them being similar to those described by Eiger (1909), Schwartz (1899) and Schurawlew (1928).



Text-fig. 2. Standardized diagram of atria and inter-atrial septum with left pre-caval vein, with ganglion fields shown by shaded areas.

A. Epicardial ganglion fields (Text-fig. 2A)

- (1) An area surrounding the orifice of the right pre-caval vein.
- (2) The anterior part of the inter-atrial groove.
- (3) The upper part of the dorsal surface of the left atrium.
- (4) A rather narrow area on the dorsal surface of the right atrium, close to the inter-atrial groove and extending downwards and laterally towards the post-caval vein.

(5) An irregular area occupying most of the medial half of the ventral surface of the left atrium.

(6) A narrow area on the upper border of the right atrium, extending to the junction of the atrium and the auricle.

(7) An area above and lateral to the orifice of the post-caval vein. It may extend below the vein medially to the opening of the coronary sinus.

(8) A narrow area on either side of the oblique vein of the left atrium (or the left pre-caval vein); when the latter vein is present, this area continues into area 3.

(9) An area surrounding the coronary sinus as far as its opening where it may be continuous with ganglia in the lower and dorsal part of the atrial septum.

(10) An irregular area on the lower part of the dorsal surface of the right atrium, extending to the atrioventricular sulcus and, in some hearts, far ventrally towards the right auricle.

(11) An area around and between the openings of the pulmonary veins, but mainly situated medially to the right veins.

(12) An area comprising the dorsal part of the inter-atrial groove; in most hearts it is interrupted near its middle.

(13) An irregular area on the ventral surface of the right atrium; it may extend to the right auricle.

Although the above areas are fairly well defined in many hearts, in some, adjacent areas may be continuous, e.g. area 1-4; areas 4 and 7; areas 6 and 13, 6 and 12.

B. Ganglion fields in the atrial septum (Text-fig. 2B)

These comprise four irregular fields which are not precisely separated from each other; all are situated between the muscle bundles of the septum.

(1) The upper ventral part of the septum, extending about three-quarters of the way downwards towards the A.V. junction.

(2) An area just below the middle of the septum, immediately below the fossa ovalis.

(3) The upper dorsal part of the septum above the fossa ovalis.

(4) The lower dorsal part of the septum, just above the entrance of the coronary sinus.

OBSERVATIONS

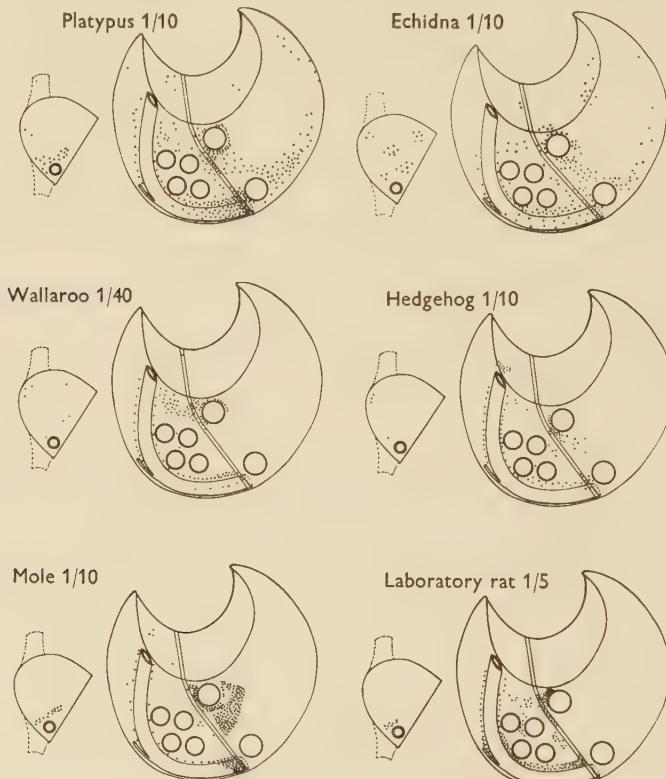
The topography of the ganglia

The majority of the atrial ganglia in all the specimens are situated in the subepicardial connective tissue (Pl. 1, fig. 1), an observation similar to that made in the case of the ventricular ganglia by Davies *et al.* (1952). The topography of the subepicardial ganglia and of those in the inter-atrial septum have been summarized for the different animals in the table (Table 1), in which the presence or absence of ganglia is shown in the columns under the headings of the numbered ganglion fields, for each animal of the series. The finding of very numerous ganglia in some sites is also indicated by the number of +'s. The positions of the ganglia under the epicardium and also in the septum are shown in the diagrammatic reconstructions

Table 1. Distribution of ganglia in atria

(Text-figs. 3-5) in which they are represented by dots, as already described. The pig and the horse are excluded from these diagrams because, as these hearts were examined in separate small blocks, it was impossible to give an accurate reconstruction of their ganglia, comparable to those of the other hearts of the series, which were sectioned whole.

Subendocardial ganglia are extremely rare; in most of the hearts none were found in this situation. The human infant was the exception to this (Pl. 1, fig. 2). In the myocardium, however, ganglia are frequently observed, in contrast with the findings

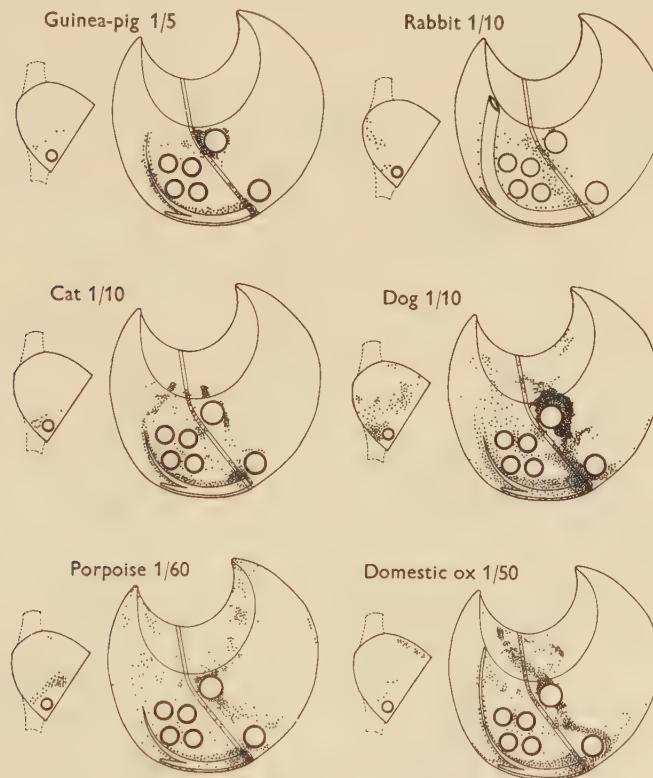


Text-fig. 3. Standardized diagrams with the ganglia plotted in.

of Davies *et al.* (1952), who saw none in the myocardium of the ventricles in any heart they examined. Intra-myocardial ganglia are present in all the hearts of this series, but, like subepicardial ones, their number varies from one Order to another. The dog is especially noteworthy in this respect, having very numerous ganglia in various places in the muscle, mainly of the right atrium (Pl. 1, fig. 7), particularly the upper part of its wall, both ventral and dorsal. Included among intra-myocardial ganglia are those of the inter-atrial septum, already mentioned.

Ganglia are associated particularly with the nodes of the conducting system. Their presence near the sinu-atrial node is a constant feature in all the hearts, and in some species the ganglia are very numerous, as in the rhesus monkey, the dog, sheep and

the calf (Text-figs. 4, 5; Pl. 1, fig. 8). In the monkey a large ganglion was observed lying on the superficial surface of the node (Pl. 1, fig. 1), and in the calf several small ones between the ordinary myocardium and the node (Pl. 1, fig. 8). Ganglia related to the s.a. node are usually close to it, often at the very edge of the nodal tissue. They are numerous near the upper end of the node in most hearts, but often extend beyond its upper and lower limits. Ganglia and isolated nerve cells are often found just above the s.a. node in relation to the superior vena cava, but they do not extend upwards in the wall of this vessel. Although ganglia at the edge of the node may be



Text-fig. 4. Standardized diagrams with the ganglia plotted in.

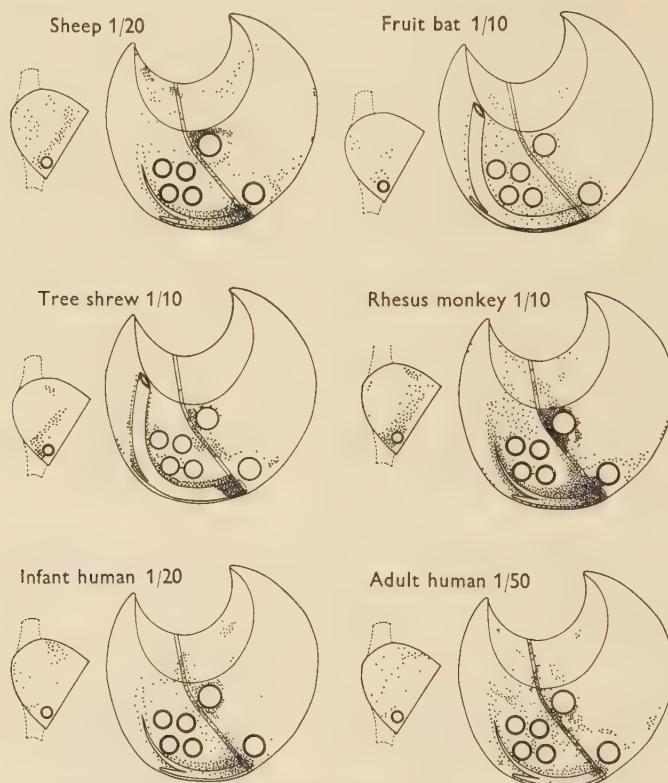
embedded in connective tissue which may pass for a very short distance between the nodal fibres, it is not very common to find ganglia actually in the substance of the node, but they do occur occasionally in this situation in man (Pl. 2, fig. 14), the calf and the porpoise.

In relation to the atrio-ventricular node (A.V. node), the commonest position of ganglia is dorsal to it, often near the opening of the coronary sinus, but they are also found alongside the node, as in the porpoise (Pl. 1, fig. 3), and very frequently above it in the lower part of the septum, in septal field 2 (Text-fig. 2). It is exceptional to find nerve cells within the A.V. node, but they were observed in this position in the rhesus monkey, the porpoise, and the calf.

Ganglia frequently accompany the atrio-ventricular bundle, being observed usually in the connective tissue close to it, and only occasionally among the bundle fibres, in which situation they were seen in calf and porpoise.

Analysis of the distribution of ganglia in the different Orders

In various animals ganglia have been observed in widely scattered parts of the atrial surface, but they are localized in the majority to certain limited areas, and the ganglion fields show the sites of their occurrence in the animals of this series.



Text-fig. 5. Standardized diagrams with the ganglia plotted in.

Ganglia are present in all the animals in four situations, namely, round the opening of the right pre-caval vein (field 1), in relation to the oblique vein of the left atrium (or left pre-caval vein) (field 8), alongside the coronary sinus (field 9), and in some parts of the inter-atrial groove (fields 2 and 12). In these positions they vary from a very large number in the dog and the domestic ox (Text-fig. 4) to only a few in the rabbit, the hedgehog, and the wallaroo. These four main ganglion positions are common to all the animals of the series, regardless of Order, the differences being only quantitative. Differences between one Order and another, and similarities in members of the same Order are observed with regard to the occurrence of ganglia in the other fields. For example, the rat and the guinea-pig have no ganglia on the

ventral surfaces of either atrium (Text-figs. 3, 4) (fields 5, 13), the ventral inter-atrial groove (field 2), nor in relation to the post-caval vein (field 7), contrary to Meiklejohn (1913, 1914), who found scattered groups round the entrance of this vessel. The long chain in the external wall of the left auricle described by her is probably the chain accompanying the left pre-caval vein (field 8). A similar distribution occurs in the rabbit. The mole and the hedgehog have each a few ganglia on the upper part of the ventral surface of the left atrium (field 5) (Text-fig. 3). Of the Ornithodelphia, the platypus and the echidna resemble each other in the disposition of ganglia, with a few on the ventral surface of both atria (Text-fig. 3) (fields 5, 13), and none in the ventral inter-atrial groove (field 2). A further resemblance is that both have a chain of ganglia leading from the right pre-caval vein to the lateral side of the post-caval vein (field 4), the only other animals in which this is present in well-marked form being the ox and the porpoise. Both representatives of the Ornithodelphia also have a chain of scattered ganglia on the lower part of the dorsal surface of the right atrium (field 10), extending out to the base of the auricular appendage. In the Artiodactyla, represented by sheep and ox (Text-figs. 4, 5), a close resemblance in the ganglion distribution is observed in the occurrence of ganglia on the ventral surfaces of both atria (fields 5, 13), and in the ventral inter-atrial groove (field 2), a similar disposition being found in the porpoise, representing the Cetacea. A further point of interest is that only in these three animals are ganglia observed in the auricular appendages, the most numerous being in the porpoise, in which they are present on both appendages, whereas in sheep and ox, a few are found on the left one only. Similar points suggesting a close affinity between Artiodactyla and Cetacea were noted by Davies *et al.* (1952) in the ventricular disposition of nerve cells.

With regard to the Primates (Text-fig. 5), the rhesus monkey has an extremely dense concentration of ganglia round the pre-caval vein (field 1), the oblique vein of the left atrium, and the coronary sinus (fields 8, 9). There is a close resemblance between the distribution in monkey and that in man, in that they have scattered ganglia on the ventral surfaces of both atria (fields 5, 13), the ventral inter-atrial groove (field 2) and a few near the base of the left auricular appendage, in the region between the latter and the atrium proper.

There are also individual variations among members of the same Order, as noted by Schurawlew (1928). In the present series, in man for example, the infant heart has no ganglia between the openings of the pulmonary veins (field 11), where there is a chain in the adult (Text-fig. 5). None were observed on the ventral surface of the left atrium (field 5) in the infant, except a few to the left of the inter-atrial groove (field 2), whereas some scattered ganglia were noted in the former situation in the adult, including a few on the ventral part of the base of the auricular appendage. In the human adult, scattered ganglia were seen on the lower part of the dorsal surface of the right atrium (field 10); a chain of ganglia leads from the superior vena cava to the inferior vena cava (field 4), but these are not present in the infant.

With regard to septal ganglia, wide differences are observed in their disposition in the various Orders. It is noteworthy that ganglia bear no constant relation to the fossa ovalis. As was seen in the case of the epicardial ganglia, there are variations in distribution in individuals of the same Order in addition to variations between

individuals of different Orders. For example, among the Carnivora, the dog has very numerous ganglia in all fields of the septum, whereas the cat has only a few in the lower and posterior part (field 4). In the Artiodactyla septal ganglia are very numerous in the sheep and pig, but the ox has comparatively few in this situation, and they are also sparse in the porpoise, representative of Cetacea. Among the Primates, the rhesus monkey has a large concentration of ganglia in the lower part of the septum (fields 2, 4), and in the anterior part (field 1), while the human, both infant and adult, has much fewer, mostly scattered in the upper part (fields 1, 3). The tree shrew has a septal disposition resembling that of the rhesus monkey, with ganglia in fields 1, 2 and 4, the latter having also a small group in field 3 (Text-fig. 3). In the Insectivora, mole and hedgehog, and in the Rodentia, rat and guinea-pig, only a very few ganglia are present in the septum. While the two rodents resemble each other in the septal disposition of the ganglia (Text-figs. 3, 4), the hedgehog (Text-fig. 3) has only a few in field 3, and one in field 4, whereas the mole has a group of ganglia in field 4 only. In the Ornithodelphia (Text-fig. 3), the platypus has a few ganglia in fields 3 and 4, while the echidna has groups in all septal fields. It is seen therefore that differences in the septal disposition of ganglia between members of one Order and another, and between members of the same Order, do exist, but have no constant relation to species.

Structure of ganglia

The structure of the ganglia varies in different animals, as also to some extent does the type of cell. Large ganglia, each of which passed through a large number of sections, were found to contain up to 200 cells (Pl. 2, fig. 9).

Ganglia of intermediate size, in the fatty tissue of the subepicardium, are either isolated, with several layers of connective tissue surrounding them, or else—most commonly—they are placed on the course of a nerve trunk. In this case, they appear as swellings on the course of nerves, in the form of small fusiform enlargements. Very small ganglia are attached to the sides of nerves. Such appearances were observed by Davies *et al.* (1952), in the ventricles of Artiodactyla and Cetacea. Other very small ganglia often give the appearance of being completely isolated, when they are only connected by a number of very small nerve fibres. Sometimes ganglia may be formed by only one or two nerve cells on the course of a nerve. In many cases completely isolated single nerve cells are found in the connective tissue, often between the muscle fibres (Pl. 1, fig. 4c). These are in the fine nerve plexuses, which run in the deeper layers of the subepicardium and between the muscle fibres.

The structure of the ganglia varies somewhat with the size. The large ones have a fairly thick connective tissue capsule, which passes into their interior, giving a supporting framework to the nerve plexus and forming delicate additional capsules to the nerve cells. Smaller ganglia have a much thinner connective tissue capsule, which in some cases appears to be completely absent. Within the ganglion, nerve cells may be scattered more or less evenly through their substance, or they may be confined to the periphery. The former arrangement is always the case with the large ganglia, but the small ones have both arrangements. The cells may be separated by a little distance from each other, or in other ganglia they are packed tightly together,

either throughout the ganglion or round the periphery. In the latter case the cells are often seen to be touching so closely that sometimes two cells may be mistaken for one large one.

The cells show great variation in size and shape, and a number of different types have been distinguished according to their form, the number of processes and their staining reactions. As stained with haematoxylin and eosin, some cells appear very obviously dark, while others have a pale cytoplasm. The same distinction is seen with silver staining, but not so clearly with van Gieson. Such staining distinctions were observed by Blair, Bacsich & Davies (1935) in spinal ganglia. The pale stained cells may be very large, medium-sized or quite small in the same animal. In these cells the cytoplasm has a fine reticulum and is evenly filled with fine granules. The nucleus is vesicular, round or slightly ovoid, with a darkly stained, thin nuclear membrane. This membrane is slightly rough, and may have several round or pointed processes, projecting for a short distance into the surrounding cytoplasm (Pl. 3, fig. 18). Often there is a shallow groove passing transversely across the nucleus, giving the appearance, sometimes, of two separate nuclei (Pl. 3, figs. 15, 16). There is usually one darkly stained nucleolus, which may be homogeneous, or may be composed of a number of short rods, arranged like the points of a star. Sometimes there is a pale area in the centre of the nucleolus with an inner dark granule like a second nucleolus within the first (Pl. 3, fig. 18). In addition to the nucleolus there are usually a number of darkly stained chromatin granules. It is very rare to find a second nucleolus.

The shape of the large pale cells varies. They may be spherical, like posterior root ganglion cells, but most commonly they are ovoid or pear-shaped, with one rounded end slightly narrower than the other. In cells of the latter shape, the nucleus is close to the wall at the wide end, being nearer to one end in most cells. The pale cells usually have one thick process emerging from the narrow end, and a number of very slender processes arising from various points scattered over the surface, ranging from a few to a dozen or more. The large process usually divides soon after leaving the cell, and these branches may again divide soon. These fibres can, in the silver preparations, often be traced for some distance, until some of them leave the ganglion. Some branches of this process may be traced to form contact with other nerve cells in the ganglion, most commonly the dark cells, or they may become lost in the plexiform arrangement of fibres in the ganglion. The short processes either join the general plexus of the ganglion, or else they become lost in a plexus which surrounds the body of the cell, the pericellular plexus. The cells with only one process, unipolar, or pseudo-unipolar cells, which are rather rare, usually belong to the large pale cell type.

Small pale cells and those of intermediate size have appearances similar to those described for the large ones. It is possible that the small cells may really be large ones in which the section has passed through one end, that containing the nucleus, because in this type of cell the nucleus often almost fills the cell.

The dark cells are of two types, large and small. Their cytoplasm is filled with darkly stained granules, so that the nucleus is almost completely obscured. The latter is round or slightly ovoid, deeply stained with one very dark nucleolus. The dark cells have a much bigger variation in shape than the pale ones. They are

practically always irregularly angular, so that they may be triangular and pointed, quadrilateral or elongated and narrow, but occasionally they may be ovoid or pear-shaped. A thick process usually rises from a sharply pointed angle of the cell body. There are also a number of slender processes arising from various points scattered over the surface of the cell. The proportion of dark cells to pale cells varies in different ganglia, from equal numbers to a majority of 2:1 pale cells to dark ones.

The nerve fibres within the ganglion form a plexus of non-myelinated nerve fibres, in which many myelinated fibres are seen in most ganglia, being more numerous in the large ones. Some of the myelinated fibres may be passing through the ganglion (Pl. 3, fig. 19), or may be ending by making contact with the cells. In a few cases it was possible to see processes of cells become myelinated, but most frequently the acquisition of a myelin sheath does not occur until some distance from the cell. In the majority of ganglia the interior is filled with a dense plexus of non-myelinated fibres with a varying number of myelinated ones, as stated above. Sometimes, however, the ganglion is so filled with tightly packed cells that any plexus present must be extremely delicate.

Capsular cells of various types are found surrounding most of the nerve cells. Some of the latter have a complete capsule of supporting cells, with dark nuclei, such cells being thick and cubical, or quite flat (Pl. 2, fig. 12c). In some cases it is possible to see that where the capsule is not complete, these capsular cells give off a number of processes. There seems to be no uniformity about these cells. In some ganglia the large pale cells have a complete capsule, whereas in others in the same animal similar cells may have only one or two capsular cells, some none at all. It is possible that in some cases the capsular cells are only present on one side of the cell and spread out on it forming an extremely thin layer. These cells stain well with haematoxylin but they are equally visible in the silver stained preparations. The large pale round or ovoid cells most often have complete capsules, while the darkly stained cells usually have incomplete ones. Isolated nerve cells usually have a few capsular cells, but the capsules are not complete (Pl. 1, fig. 4c). The fusiform cell in fig. 5, Pl. 1, has no capsular cells.

The majority of these nerve cells are multipolar and the processes pass between the capsular cells (Pl. 2, figs. 10, 11), where the capsule is more or less complete. The ganglia are well supplied with blood capillaries which are usually plainly visible among the fibres of the plexus.

There seems to be no significant difference in the structure of ganglia in the animals of the different Orders. The impression is that in smaller hearts, whether adult or infant, the nerve cells themselves are smaller and more frequently round or oval. For example, the ganglia and cells in the small puppy heart are very similar to those of the adult fruit bat and the rhesus monkey. With the larger hearts, the ganglia of the calf resemble those of the human. In the porpoise, however, there is a marked difference in shape. In this animal the majority of the nerve cells are angular and rather flattened, the round or ovoid type being rare (Pl. 1, fig. 3g; Pl. 3, fig. 17).

In all the animals examined the majority of cells are multipolar, a few bipolar, while recognizable pseudo-unipolar cells are extremely uncommon.

DISCUSSION

There is an astonishing disparity in the previous results of research on the topography of the cardiac atrial nerve cells. To consider first the subepicardial ganglia, a large number of the earlier authors found them scattered over the surface of both atria, and indeed, among the later authors, Glomset & Glomset (1940*a, b*) observed nerves and ganglion cells over the entire left atrium, but this was the case in none of the animals of the present series. Jacques (1894, 1895), Eiger (1909), Lissauer (1909), and others, stated that ganglia only occur on the subepicardial nerves. Many ganglia in this series were undoubtedly attached to the nerves, both large and small, of the subpericardial plexus but, nevertheless, numerous ganglia were observed in the subpericardium which were quite isolated and not attached to these nerves by obvious connections.

The areas of the atria described as ganglion fields by earlier workers are much less definite than those of the present authors. Krehl & Romberg's field (1892) was an area extending up to the right to the opening of the superior vena cava, downwards to the inferior vena cava, across to the opening of the left pulmonary vein, and from there up to the pericardial reflexion at the transverse sinus, and downwards almost to the A.V. groove. This would include most of the dorsal inter-atrial groove and an area on either side. The ganglion field of Schwartz (1899) was a limited region on the posterior atrial wall to the left of the inter-atrial septal groove, bounded by the posterior end of the auricular appendage and below by the transverse coronary sinus. Eiger (1909) described his field as being bounded on the left by the left pulmonary veins, on the right by the right pulmonary veins, superiorly and anteriorly by the pericardial reflexion of the transverse sinus, below and behind by the A.V. groove. Schurawlew (1928) considered that the nerve ganglia of the heart were grouped into precise regions to form ganglion fields. He had seven, of which the first two were really associated with the pulmonary artery and do not come within the classification of the present study. His field D.1 corresponds to field 4 of the present study; his D.2 and B.2 would be included in field 1: his field C, corresponds to field 8: and B.1 includes field 6. He noted individual variations in the ganglia in the same field in different animals, even in the same species, and this agrees with the findings of the present authors.

The presence or absence of ganglion cells in the hearts of various animals was the object of much of the early research. Most later authors who studied the topographical distribution of the nerve cells in the heart came to the conclusion that their arrangement was the same in all groups of mammals and was similar in man. For example, Lim Boon Keng (1893) considered the distribution of the cardiac ganglia in the dog to be the same as in the horse and the ox, and Perman (1924) maintained that in mammals as in man, ganglia were present on the dorsal wall of the atria and on the bases of the great arteries, but with his method, many ganglia of microscopic size would escape his observation. A few authors, however, noted a difference in their findings in different animals. Woppard (1926) observed ganglia on the anterior surface of the left auricle, in which situation they were found in most of the animals of the present series, although in varying numbers, but not in wallaroo, rat, guinea-pig, rabbit or tree shrew. Woppard noted slight differences in the arrange-

ment in the dog as compared with that of the cat. In the former, ganglia occurred, according to him, in clumps on the posterior surface of the left auricle near the inter-atrial septum, whereas in the cat he found six distinct groups of nerve cells in that situation. As can be seen in Text-fig. 4, ganglia are very numerous in the dog in all fields, and they are almost as widespread in the cat. In the dog the occurrence of ganglia in clumps was not observed by the present authors. Nonidez (1943) also noted some differences. Perinodal ganglia, according to him, which were present in calf and sheep, were absent in dog and monkey, although in the present studies such ganglia were numerous in both the latter animals also. Nonidez found ganglia in the inter-atrial septum near the node.

Many workers have reported the presence of ganglia in the inter-atrial septum, either in the connective tissue between the right and left halves, or among the muscle bundles. The earlier workers, too numerous to mention, who have observed ganglia in this situation, include Tawara (1906) in the sheep and human, Keith & Flack (1907) in a number of mammals, and Meiklejohn (1913) in the monkey and the rat. It should be noted, however, that a number of these authors included the inter-atrial groove as part of the septum. Care has been exercised in this study to distinguish ganglia in the grooves from those actually within the muscular septum, and only these latter are regarded as being septal ganglia. Iwanowsky (1876) found ganglia on the muscle bundles surrounding the fossa ovalis in the human. Wolhynsky (1928), in the calf, observed ganglia in the superficial layer of the fasciculus limbicus inferior on the right side of the inter-atrial septum. In the present study ganglia were not seen in these situations and, in fact, the region surrounding the fossa ovalis was as a rule quite devoid of ganglia. Francillon (1928), in man, maintained that the ganglia were in the connective tissue pushed in between the muscle bundles of the inter-atrial septum. Later authors who observed ganglia in this position include Blair & Davies (1935), who noted them in the lower and back part of the septum in calf and man, whereas, in the corresponding animals in this study they were found mainly in the lower front part. Nonidez (1943) saw a few ganglia in the monkey just above the A.V. node, in which situation a large number were found in this animal by the present authors. Others who observed ganglia in the septum were Walls (1942, 1943) and Baird & Robb (1950).

Apart from ganglia in the inter-atrial septum the presence of ganglia within the myocardium, actually among the muscle fibres, has been questioned for years. In the present series of animals, as mentioned above, ganglia within the myocardium are quite numerous in the dog, in various parts of the atrial wall, mainly the upper part of the right atrium. Isolated nerve cells, as well as ganglia, both large and small, are found among the muscle fibres. In other hearts intra-myocardial ganglia are much less common. Many of the earlier workers, as noted by Francillon (1928), observed ganglion cells among the muscle fibres of the myocardium in man and a number of mammals, but this was denied by others, including Woppard (1926) and Francillon himself. However, Pannier (1935) in the cat, and King, A. B. (1939) in the rat, reaffirmed the existence of ganglion cells among the muscle fibres.

Okamura (1929) observed small cells on the perimuscular plexus among the muscle fibres, but the present authors have not been able to identify cells such as he observed. They are probably similar to the interstitial cells described by Boeke

(1933-7). Tcheng (1951) noted buried ganglia between the right lateral auricular and ventricular myocardia which were continuous with the superficial ganglia in the auriculo-ventricular sulcus.

The discovery of intra-myocardial nerve cells in the atria in this study contrasts with that of Davies *et al.* (1952), who found none within the myocardium of the ventricles of any heart they examined.

Ganglia have only rarely been reported as present in the subendocardium. Iwanowsky (1876) described nerve cells in a triangle with its apex in the fossa ovalis, and these latter may have been in the subendocardium. Lissauer (1909) described them in this position in rabbit, cat and dog. King, A. B. (1939) stated that ganglia just beneath the endocardium in the rat were common, but they were not found in this situation in those animals in the present study. No other authors have observed ganglia in the subendocardium of the atria, but Davies *et al.* (1952) found them in this position in the ventricles in Artiodactyla. Subendocardial ganglia were observed in the atria extremely rarely in this work, but a few were present in the infant human (Pl. 1, fig. 2), none in the adult human, and none in the subendocardium of the Artiodactyla, nor in that of the porpoise.

The close relation of ganglia to the conducting system, especially the s.a. node, has been noted by a number of authors who are mostly in agreement with the findings of the present study. Some disparities were observed by a few workers.

Nerve cells have been found within the s.a. node by Fahr (1909) occasionally in man, and by Oppenheimer & Oppenheimer (1912) in the sheep. Perman (1924) was unable to find them in this situation, and Francillon (1928) emphasized that in man ganglion cells were never found within the s.a. node. Copenhaver & Truex (1952), however, observed numerous ganglion cells within the connective tissue of the nodal area in the sheep, and also within the s.a. node of the human specimen, but not in the monkey. In the present study they were noted in this position in the human infant (Pl. 2, fig. 14), but not in the adult; they were also seen in the porpoise but not in other animals. Davies (1931), however, observed a number of nerve cells actually in the s.a. node of both platypus and echidna, but the present authors have not been able to confirm this. In their specimens, ganglia were limited to the edge of the nodal tissue. It is of interest that both Shaner (1929) in calf embryos, and Walls (1947) in human embryos, noted the early appearance of nerve cells in the area of the future s.a. node.

With regard to the atrioventricular node, in common with most, the present authors have found ganglia regularly near it or in its neighbourhood, but not actually among the specialized tissues, with the exception of the calf and the porpoise, in which small ganglia were observed deep within the node. In some of the other animals, ganglia were occasionally found in the connective tissue at the edge of the node. Wilson (1909) noted ganglion cells in the a.v. node in the Artiodactyla, and Fahr (1909) and Blair & Davies (1935) saw a few nerve cells buried within it in calf and man. In the present series of animals, a few ganglia were found alongside the a.v. node in some cases, while in others they were noted above the node, in the lower part of the inter-atrial septum, where they were observed by Nonidez (1943) in dog and monkey. The commonest situation, however, for ganglia related to the node, is posterior to it in the lower part of the inter-atrial groove, where numerous ganglia

were found in most hearts. Stotler & McMahon (1947) observed numerous ganglia near the artery round the A.V. node in man, but this was not found to be a regular feature in the present series.

The A.V. bundle is included within the scope of this work, but not the limbs, which more properly belong to the ventricle, and were dealt with by Davies *et al.* (1952). It is noteworthy that in the Artiodactyla, e.g. the calf, and in the Cetacea, e.g. the porpoise, ganglia were found within the A.V. bundle where they were not seen in other animals. Fahr (1909) was unable to find any nerve cells in the bundle in man, but Engel (1910) saw numerous nerves and ganglion cells in this situation in Artiodactyla. According to de Witt (1909), nerve cells and fibres were numerous near the bundle in the sheep and calf, but only in the latter were they noted within its meshes. Meiklejohn (1913) described a few small ganglia in the bundle in the guinea-pig, but the present authors were not able to confirm this. Blair & Davies (1935) observed small groups of nerve cells in the A.V. bundle in the bovine heart, while Stotler & McMahon (1947) stated that no ganglia were seen near the bundle or its branches in man.

With regard to the question as to which ganglia in mammals correspond to those found in the frog, it is difficult to find any true comparison. Ludwig's ganglion (1848) was a collection of ganglion cells in the frog in the course of the nerves to the atrial septum. Remak (1844) described a group of ganglion cells on the nerves which penetrated the atrial septum, while Bidder's ganglion (1852) lay in the lower part of the atrial septum on the entrance of the nerves into the ventricular septum. According to Flack (1909) groups of ganglia were found in vertebrates often in the 'sinu-auricular junction' in the atrial septum, and in the coronary sulcus, which correspond to Remak's, Ludwig's and Bidder's ganglia respectively, while Aschoff (1909) considered that the ganglia lying on the superior vena cava corresponded to Remak's ganglion, and those which lie on the dorsal wall of the atrium and near the A.V. node to Ludwig's ganglion; and those which lay on the base of the aorta and the pulmonary artery corresponded to Bidder's ganglion. The present authors noted, as also did McFarland & Anders (1913), the small size and disseminated character of the ganglia in the human and the mammalian heart. The latter authors made numerous attempts to find the homologues of Remak's ganglion in the inter-auricular groove, and of Bidder's ganglion in the interventricular groove at the A.V. junction with little success.

The ganglia in the human and mammalian atria are much more diffusely arranged than are those of the frog, and, as Perman (1924) and others have described, the ganglia are mostly in the subpericardial nerve plexus. Francillon (1928) was more definite than Perman, and considered that they compared as follows for the human: the ganglion of Remak in the frog corresponds to the ganglia in the region of the sulcus terminalis and on the wall of the left atrium. Ludwig's ganglion consists of the ganglia in the region of the aorta and the pulmonary artery and in the commencement of the coronary plexus and in the coronary sulcus. Considering the manner in which the cardiac nerves spread out over the heart it would seem to be impossible to identify ganglia in the frog with those in the human.

As the cardiac nerves, sympathetic and vagus, form a plexus with one another, it has long been a question as to whether some ganglia might be mixed, or exclusively

either vagus or sympathetic in their connections. It has often been stated that the S.A. node is innervated by the vagus, and the A.V. node by the sympathetic, following the work of Marchand & Meyer (1912), and attempts have been made by various workers to reach a decision on this point. Woppard (1926) found no change in the cells after removing the stellate ganglion and, therefore, believed that all cardiac nerve cells belong to the para-sympathetic system. Degeneration studies have also been made by Soler (1953, 1954, 1956) and from these he concluded that the ganglia of one or other side of the heart receive connexions from the vagus and sympathetic of both sides. Soler (1956) and Corzo (1956) described two types of ganglia according to the staining of the cells with silver. The darkly stained ganglia they called argentophil, while the others which were pale with silver staining they designated argentophobes. Although the difference in staining of individual cells with silver was noted by the present authors, it was usually found that the ganglia were mixed and contained both types of cell. Corzo noted after degeneration studies following unilateral and double vagectomy and stellectomy in the cat that the argentophil type of ganglia was connected with the parasympathetic system, while the argentophobe type was connected with the sympathetic. In the present work, the ganglia contained cells of different types, and it appeared to the authors that the pale staining large cells in their sections were equivalent to Corzo's argentophobe type of cell, and that they closely resembled the large cells of the posterior root ganglia. They conclude that ganglia are generally connected to both vagus and sympathetic, and that they contain both sympathetic and parasympathetic elements.

A number of earlier authors observed differences in the nerve cells in the atrial ganglia from those in the ventricles. Vignal (1881) found unipolar cells in the ventricles and multipolar cells in the atria in the cat, dog, sheep, guinea-pig and man, whereas in the rabbit he noted ganglia in the atria with both types of cell, but in the ventricles they were exclusively unipolar cells with one nucleus.

Davies *et al.* (1952), in the ventricles of Artiodactyla and Cetacea, found that the epicardial nerve cells were mostly bipolar and unipolar. In the atria, in the present series, most of the nerve cells in the epicardial ganglia are multipolar, and this also applies to ganglia elsewhere in the atria. The same appearances of bipolar and unipolar cells observed by the above authors were noted in the present work, although in the atria these cells are rather rare, a point of distinction from the ganglia which they described in the ventricles.

Earlier workers distinguished the types of ganglion cells by their shape, number and type of processes, and the number of nuclei. In the present studies no cells with double nuclei were found, such as were described by Ranvier (1875) in his type 1, and Vignal (1881), Kasem-Beck (1884) in the rabbit, and by Kulesch (1901). Michailow, S. (1912) sometimes observed isolated ganglion cells with two nuclei in rabbits, horses and apes; he considered that nuclei are single, rounded or regularly oval in the majority of cases. Cells which appeared to be in process of division as described by Michailow, S. (1912) were not seen by the present authors.

Kasem-Beck (1884), in the rabbit, maintained that cardiac nerve cells had an oval form with only one process. His, Jr. (1891), in the hearts of embryos, observed that the nerve cells had only one process; it is to be noted however that these were young embryos and the nerve cells would be in the neuroblast stage. Berkley (1893), in

the mouse and the rat, found the nerve cells to be of medium size, both bipolar and multipolar. The processes of the cell were not always limited to two, but he considered them all as bipolar cells. Jacques (1894), in rats, mice and other small mammals, was of the opinion that the majority of cells were multipolar, but some bipolar cells occurred and, rarely, a cell with one process which branched. All the cell types lay in one and the same ganglion. Schmidt (1897), in the mouse and rabbit, never saw bipolar cells, considering such appearances as simple varicosities. He found multipolar nerve cells in the myocardium of the atrium and ventricle with an axis cylinder going to the nerve stems. Noc (1899) considered that cardiac ganglia comprised a varying number of multipolar and unipolar cells, and that their structure was almost identical with the nerve cells of the spinal cord.

Lissauer (1909) described the ganglion cells as very large, round, oval or pear-shaped, with a cytoplasm which was faintly granular, and containing one or two large round clear nuclei, and dark nucleoli, of which there were usually one, and often two. He was only able to see a process in isolated cells. Cells with two nuclei were also seen by Michailow, S. (1912) and Michailow, M. (1898-9), but such cells were not found in this work although many nuclei have a shallow transverse groove, which gives the appearance of a dividing or a double nucleus. This could not be a stage in normal mitosis, because the nuclear membrane is still intact. It was possible to find only occasionally in this study fenestrated cells, as described by Michailow, S. (1912), but many nerve cells contained pigment as he observed.

With the staining technique used by the present authors, by which the shape and details of the body of the cell and the nucleus can be seen, and the processes distinguished with varying degrees of sharpness, the latter are not seen as clearly as in the studies of Dogiel (1899) and Michailow (1912) with methylene blue. On this account it is difficult to identify the former's three cell types and the latter's five types with the four types observed in this study (Pl. 2, figs. 12, 13). Both Dogiel and Michailow distinguished their cells mainly by the number and types of processes. Dogiel's type 1 cell had up to sixteen dendrons and one thick axon arising from a thick cone, which latter distinguished it from other types. The cell was round, oval or angular, and usually rather small, while Michailow's type 1, similarly round, oval or pear-shaped, had one thick axon and a number of thick dendrons. These cells resemble the types of large and small pale cells observed by the present authors (Pl. 2, figs. 12, 13), which usually have one thick process and a large and variable number of small processes, but it is not possible to identify the axon with certainty. Many cells, as in Pl. 2, fig. 11, have a number of processes all of equal diameter. Michailow's type 2 is similar, but has two kinds of dendrons, namely two to nine club-shaped ones and one to four flattened dendrons with condensed endings. Cells with dendrons of this type have not been found in this study, possibly because the processes could not be followed to their termination. Dogiel's type 2 and Michailow's type 3 are both similar to the dark cells, both large and small, and also include the dark fusiform and bipolar cells of the present study (Pl. 1, fig. 5). Both the above authors described unipolar cells in this group, but from the descriptions they appear to be pseudo-unipolar because the single process gave off the axon (described by Michailow as a thin flattened thread) and so are similar to the rather rare pseudo-unipolar cells in the present study (Pl. 2, fig. 13a).

Wilson (1909), in the calf and sheep, found three types of cells, unipolar, bipolar and multipolar. Woppard (1926) confirmed the findings of Dogiel (1899) and Michailow (1912). De Castro (1932), in the human heart, found cells of three types described according to the lengths of their processes, short, long and intermediate. Okamura (1929) found two types: large cells which were always multipolar; and small cells which were almost always spindle-shaped, and occurred in the plexus surrounding the muscle fibres. Blair & Davies (1935) described two types of cell in the calf and human: a larger type, with rounded form and finely stippled cytoplasm, which is pale yellow with silver; and a smaller, rather angular cell, staining darkly with silver and having a darkly staining nucleus. Similar types of cells, which were pale and dark both with silver and with haematoxylin and eosin, were noted by the present authors and form the main types seen (Pl. 2, fig. 12).

Glomset & Birge (1945) described two types of ganglia in the sulcus terminalis in man. Of these the larger groups consisted of large cells and were associated with the entering vagi, while the other groups, made up of smaller cells, were found some distance from the nerves. They observed these two types of cells in every species examined.

Walls (1942, 1943), in the hedgehog and the golden hamster, found only one type of cell, which was large and round with fine granular cytoplasm, and no cells corresponding to the second ganglion type of Kiss (1932). In the present study, however, the hedgehog heart exhibited the same types of cell as were noted in the other hearts, including cells resembling those described by Kiss in spinal ganglia. Walls (1943) also found, in association with the nerve cells in the ganglia, clumps of small dark cells which, in size and general appearance, were very similar to small lymphocytes. These cells have been observed in some of the ganglia by the present authors and it is considered that they are definitely lymphocytes, possibly indicating some focus of infection. It is interesting to note that Pančenko (1940a, b), in the lumbar ganglia, found a constant infiltration of lymphocyte cells after sympathectomy.

Many of the earlier workers made their cell descriptions from the study of embryos, and according to some recent work the discrepancies in the descriptions of cardiac nerve cells can partly be explained by age changes after birth. Walls (1947) found that at no time before birth are there two cell types in the cardiac ganglia of the human, as in the adult. The single type of the foetus corresponds to type 1 cell of Blair & Davies (1935). Lasowsky (1930) found that the cardiac nerve cells of the newborn have few processes and resemble the neuroblast type. Between the predominating mass of small, undifferentiated cells, this author observed some larger nerve cells with numerous processes. Hermann (1949) examined a series of human hearts from foetal life up to old age. He found an appearance similar to that of Lasowsky (1930) in the newborn, and observed that the change up to adult life consisted in an increase in size of the nerve cell body, and a gradually increasing complexity of the nerve processes. Conti (1948) demonstrated that the nerve ganglion cells of the heart of man are subject, during a long period of life, to a continual remodelling of their shape, which is shown visibly by an increase in volume of the pyrenophore, by a luxuriant growth of the dendritic apparatus, and also by the appearance of paraphyses. Although a similar study has not been made in mammalian hearts, it would appear likely that changes similar to the above

described ones, but probably occurring at a faster rate, would be found. However, it was not observed by the present authors that the cells of the ganglia in the calf showed any marked difference from those of the adult cow. On the other hand, the ganglion cells of the infant human heart seem to be rounder, and less obviously multipolar than those of the adult human. Such growth changes must, therefore, be borne in mind in any description of the cardiac nerve cells. In the present study, in those Orders in which the main heart sections were obtained from a young animal, such as the puppy, comparisons have been made with sections from adult hearts.

The functions of the cardiac ganglia have naturally aroused considerable speculation among various workers. Soler (1953, 1954, 1956), for example, from his degeneration studies has concluded that the ganglia have both afferent and efferent connexions with the vagus and sympathetic. The functions of the different types of cardiac nerve cells have been thoroughly discussed by Davies *et al.* (1952), who on purely morphological grounds suggested that the multipolar ventricular nerve cells might be efferent, and the bipolar and unipolar cells afferent in function. They noted similar types of nerve cells in the atria and made the same suggestion for them. From observations in the present study it would appear that these authors noted a larger proportion of unipolar and bipolar cells in the ventricular ganglia than were seen in the atria in this series. However, it was only in Artiodactyla and Cetacea that they saw numerous ganglia in the ventricles. Other animals, not having these ventricular ganglia, must supply their ventricles from ganglia in the atria, and it is felt, therefore, that many of these, observed by the present authors in the atrio-ventricular groove would have this function, as also would many of the large number accompanying the coronary sinus. It would be expected, therefore, that in animals without ventricular ganglia, those in the lower part of the atrium would have the composition observed by Davies *et al.* in ventricular ganglia in Artiodactyla, that is, a greater proportion of unipolar and bipolar cells. It was not possible, however, for the present authors to confirm any such difference in constitution of the lower, as compared with that of the upper atrial ganglia. In any case, as the cardiac ganglia are mainly concerned with a nerve supply either afferent or efferent to the cardiac muscle and its blood vessels, the much larger volume of these tissues in the ventricles would necessarily demand a much greater proportion of the total available nerve supply. Probably, therefore, many of the atrial ganglia must supply the ventricles, others would be expected to supply the conducting system and the nodes, and the atrial muscle, while, of the remainder, some must be distributed to the venae cavae and the coronary blood system, including the sinus. To elucidate the problem of the connexions and distribution of the ganglia it would be necessary to trace all the nerve fibres from them to their destination. This presents great technical difficulties, but with a more accurate knowledge of the topography of the nerve ganglia such work may be possible in the future, especially with improvements in surgical techniques.

SUMMARY

1. Hearts from animals of a large number of Orders of mammals have been examined by histological methods, the hearts being sectioned as a whole transversely. A number of different staining methods have been used.

2. The positions of the intrinsic ganglia were plotted on to a standardized diagram for each heart, and a number of ganglion fields described.

3. The ganglia in the epicardium are found regularly in certain situations, namely, round the opening of the superior vena cava (right pre-caval vein) in the inter-atrial groove, mainly the dorsal part; the upper part of the left atrium; and a continuous chain accompanying the oblique vein of the left atrium or left pre-caval vein and the whole length of the coronary sinus. A few ganglia are found near the pulmonary veins and the inferior vena cava, on the ventral surface of the left atrium, and occasional ones on the ventral and dorsal surfaces of the right atrium. With few exceptions ganglia are never found on the auricular appendages. Ganglia are also found regularly in varying numbers within the interatrial septum, in most hearts among the myocardial fibres, and very rarely in the subendocardium.

4. Ganglia are associated with the conducting system. In all hearts they are found near both nodes and the bundle. Nerve cells have only rarely been observed within the specialized tissue of either the sinu-atrial or the atrioventricular nodes.

5. Differences in the distribution of ganglia between members of one Order and another, and individual variation within the same Order are noted.

6. The structure of the ganglia is described, and a number of different cell types have been distinguished. These are pale and dark cells, both large and small, and fusiform cells. The majority of cells are multipolar, but some bipolar cells are found and a few recognizable pseudo-unipolar cells.

7. The discrepancies in the earlier results have been discussed. An identification of the ganglia of Remak, Ludwig and Bidder with those in the mammal and man is considered to be practically impossible owing to their diffuse arrangement in these groups of animals.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Subepicardial ganglion (g.) beside sinu-atrial node (n.), in rhesus monkey. H. & E., $\times 88$.

Fig. 2. Subendocardial ganglion (a.). Infant human. Silver, $\times 200$. end.=endocardium.

Fig. 3. Ganglion (g.) at edge of atrio-ventricular node (n.) in porpoise. Van Gieson, $\times 120$.

Fig. 4. Two single isolated nerve cells (c.). *Pteropus*. H. & E., $\times 510$.

Fig. 5. Bipolar cell. Human. Silver, $\times 510$.

Fig. 6. Pseudo-unipolar cell (d.) in calf. Silver, $\times 325$.

Fig. 7. Intra-myocardial ganglion (b.) in dog. H. & E., $\times 220$. m.=myocardial fibres.

Fig. 8. Ganglion (g.) beside sinu-atrial node (n.) in calf. Silver, $\times 132$. m=ordinary atrial muscle.

PLATE 2

Fig. 9. Part of very large ganglion in rhesus monkey. H. & E., $\times 120$.

Fig. 10. Multipolar cell. Cat. Silver, $\times 510$.

Fig. 11. Multipolar cell. Adult human. Silver, $\times 510$.

Fig. 12. Ganglion showing dark staining and pale staining cells. *Pteropus*. (c.) capsular cells; (d.) large pale staining cell, (e.) dark angular cell; (f.) dark round cell; (g.) dark flattened cell. H. & E., $\times 510$.

Fig. 13. Pseudo-unipolar cell (a.). Human. Silver, $\times 570$. b.=small pale cell.

Fig. 14. Ganglion in sinu-atrial node (n.). Human infant. Silver, $\times 510$.

PLATE 3

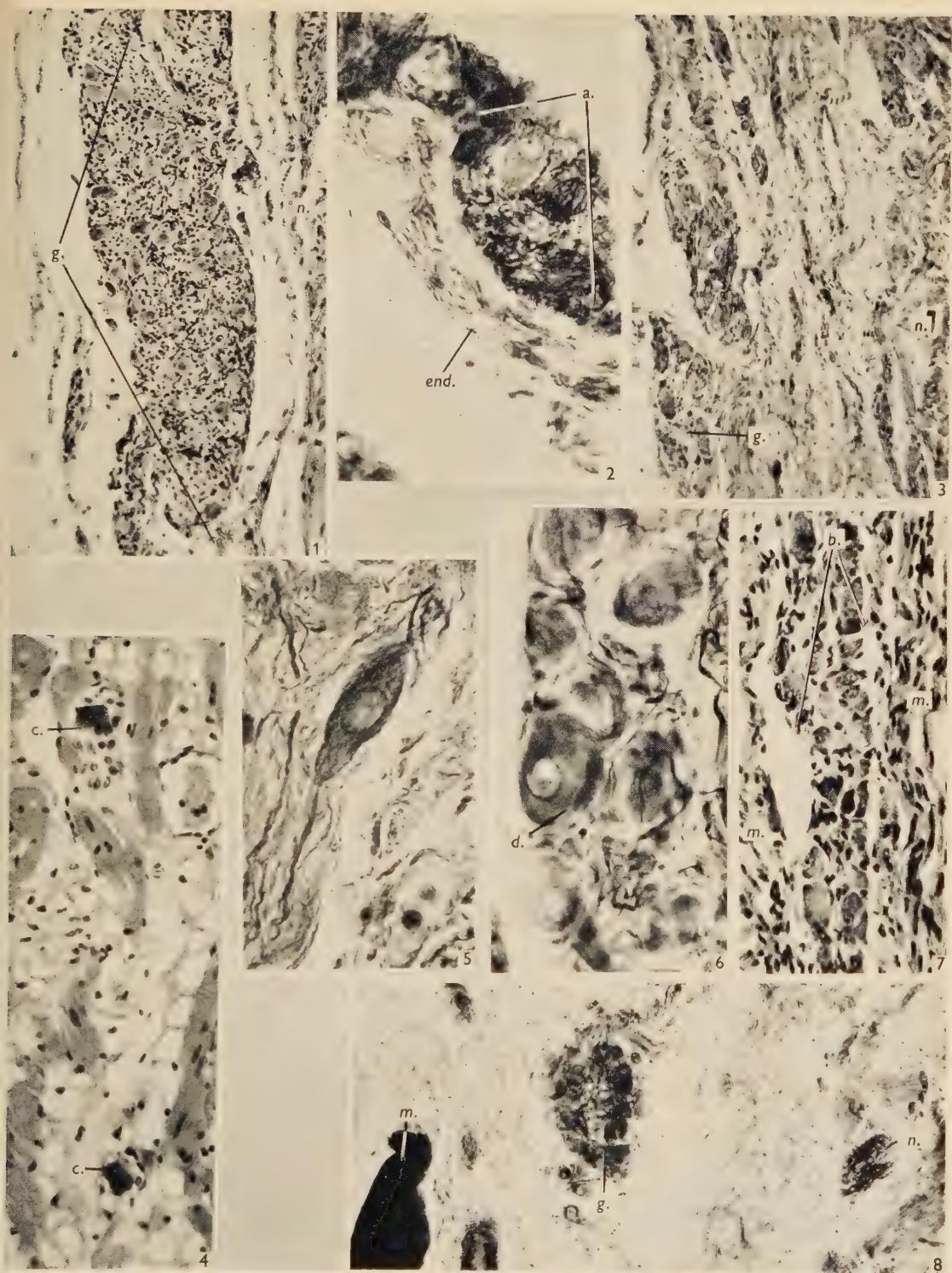
Fig. 15. Part of atrial ganglion of talpa. Cell (a.) gives the appearance of having two nuclei. H. & E., $\times 650$.

Fig. 16. The same cell as in fig. 15 under oil immersion showing a single nucleus with a shallow groove at (b.). $\times 1400$.

Fig. 17. Atrial ganglion of porpoise, showing narrow angular cells. Van Gieson, $\times 280$.

Fig. 18. Cell from atrial ganglion of rhesus monkey showing nucleus with pointed processes (c.). A dark granule is seen in the centre of the nucleolus (d.). H. & E., $\times 1400$.

Fig. 19. Atrial ganglion of rat, showing myelinated fibres (e.). Osmic preparation, $\times 570$.

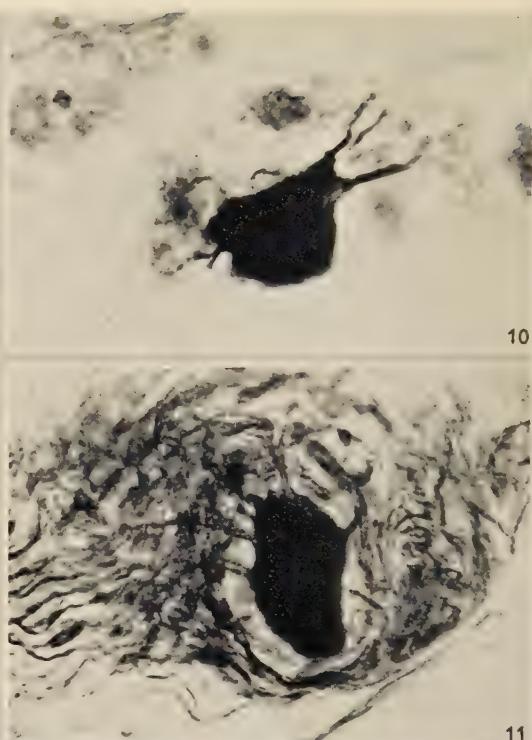


SUMMERFIELD KING AND COAKLEY—INTRINSIC NERVE CELLS OF CARDIAC ATRIA OF MAMMALS AND MAN

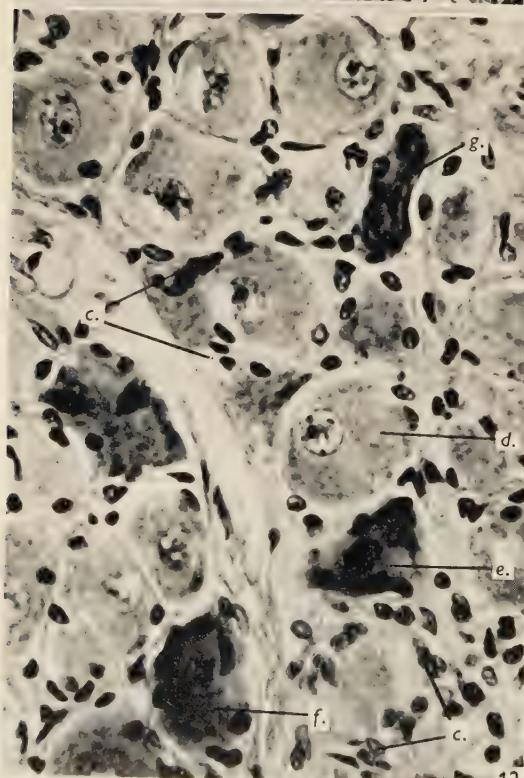
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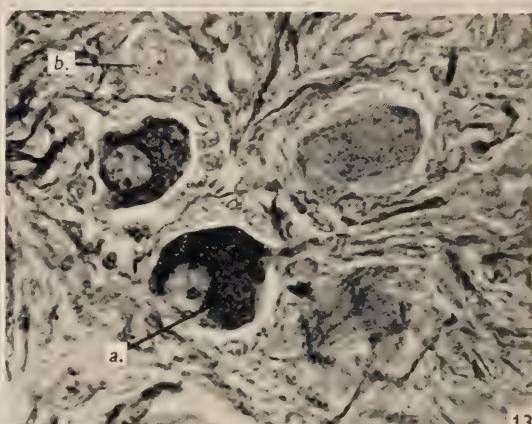
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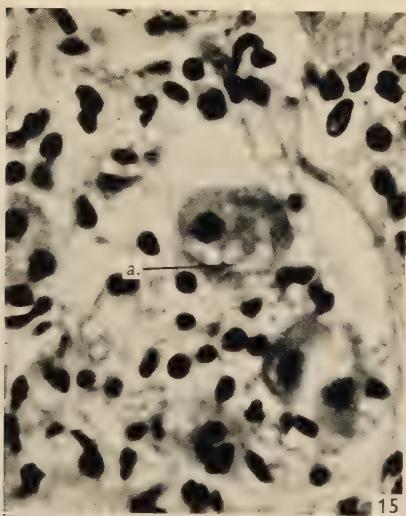
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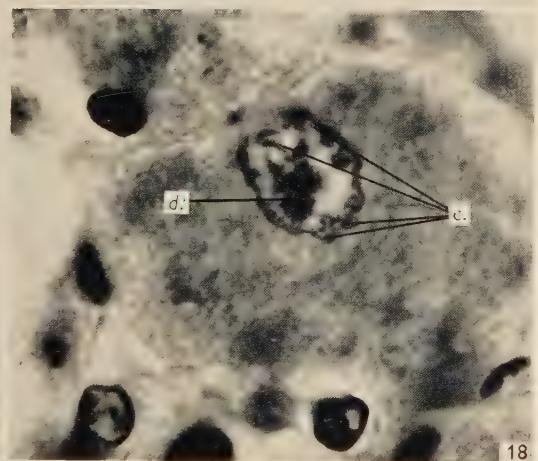
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THE DEVELOPMENT OF THE HUMAN CAUDATE AND AMYGDALOID NUCLEI

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INTRODUCTION

The literature concerning the development of the basal ganglia contains many discrepancies. They are said to be derived from a single mass in the cerebral hemisphere (Arey, 1954), from a single ridge later subdivided into two parts (Hamilton, Boyd & Mossman, 1952) or from two masses (Baxter, 1953; Frazer, 1931; Hines, 1921, 1922; Kappers, 1928, and Elliot Smith, 1919). Bryce (1908), His (1897) and Streeter (1912) considered that a single mass was formed with three anterior limbs or projections. Little is known, however, about the late stages of development.

MATERIALS AND METHODS

Twenty-six human embryos and foetuses, of c.r. lengths ranging from 7·5 to 335 mm., were used. The majority of these had previously been fixed in 10% formalin, the rest in Bouin's solution. In fresh foetuses of 265 mm. and over, 10% formalin was injected into the common carotid arteries prior to removing the brain.

Waxplate reconstructions were made from transverse sections of the forebrain of the 7·5 mm. embryo and coronal sections of the cerebral hemispheres of the 15 and 35 mm. embryos. In all other specimens, including a 25 mm. embryo, the caudate and amygdaloid nuclei were examined macroscopically by removing the medial wall and roof of the ventricle and excising part of the thalamus to complete the exposure.

RESULTS

The early formation of the basal ganglia presented as a single antero-posterior swelling in the side wall of each cerebral vesicle in the 7·5 mm. embryo (Fig. 1). This terminated anteriorly by merging with the wall of the hemisphere and did not diverge into three limbs as has been said (Bryce, 1908; His, 1897, and Streeter, 1912). The only other interruption of the surface of the forebrain cavities was a low, narrow ridge across the floor of the diencephalon connecting, through the large interven-tricular foramina, the striatal mass of each side.

In the floor of the hemispheres of the 15, 25 and 35 mm. embryos two adjacent elevations were present (Figs. 2, 3). Anteriorly these were separated by a groove which became indistinct posteriorly where they united forming a common stem extending to the posterior or future temporal pole of the hemisphere. This stem was the precursor of the tail of the caudate nucleus and the amygdaloid nucleus. In the two shorter embryos the elevations extended the same distance forward where they diverged. The medial of these, which terminated above the olfactory tubercle, was the more

bulky and bulged through the still large interventricular foramen. It was also connected to its fellow by a ridge across the floor of the diencephalon and was, therefore, considered to be homologous with the single ridge seen in the 7.5 mm. embryo. In the 35 mm. embryo (Fig. 3) the medial part of the future head of the nucleus had been outstripped by the lateral which extended further forwards and backwards,

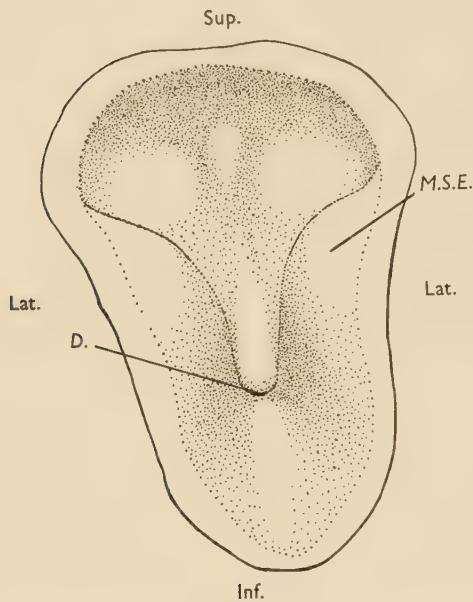


Fig. 1

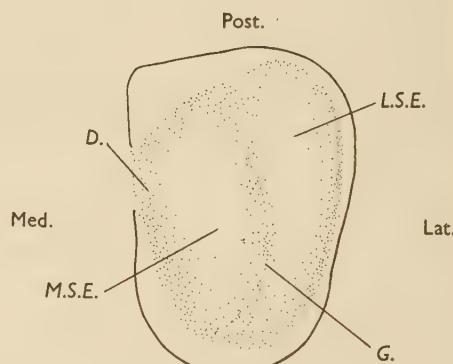


Fig. 2

Fig. 1. Reconstruction of the forebrain of a 7.5 mm. c.r. length human embryo, viewed from behind. $\times 40$. (For the key to the abbreviations used in all figures, see p. 382.)

Fig. 2. Reconstruction of the floor of the left cerebral hemisphere of a 15 mm. c.r. length human embryo, viewed from above. $\times 27$.

where it now appeared to be the major component of the tail. The medial ridge, which had only slightly increased in size, had become separated by a deep cleft from the thalamus and paraterminal body, between which it tapered through the narrower interventricular foramen. The ridge in the floor of the diencephalon, although smaller than hitherto, was nevertheless still evident as a thin strand.

In the remaining material studied, the formation of the head of the caudate nucleus was completed by the introduction of a third component between the other two. This was first seen in the 45 mm. embryo as a small tubercle in the medial side of the lateral ridge projecting into the notch, where the two parts of the head of the nucleus diverged (Fig. 4). In its subsequent growth this additional third or intermediate part outpaced the other two and, separated by a groove on each side, developed like a wedge between them. As a consequence, the lateral elevation was reduced to a thin comma-shaped strip curling around the anterior end of the nucleus and contacting its medial part over the olfactory tubercle. In foetuses about 180 mm. long the surface of the intermediate elevation became flat, but ultimately it regained

its convex form and in the 230 mm. foetus it had almost obliterated and overgrown the lateral part. In the largest of all the specimens the mature adult form of the head of the nucleus was reached, and only a faint groove separating the medial and intermediate components remained.

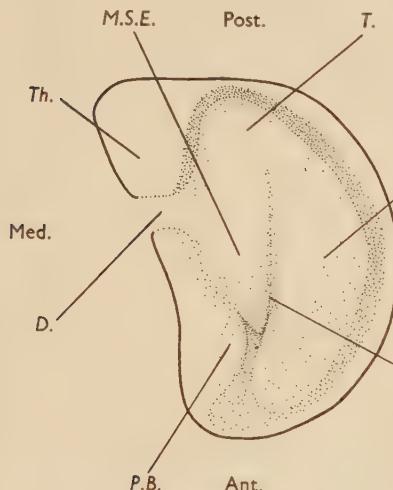


Fig. 3

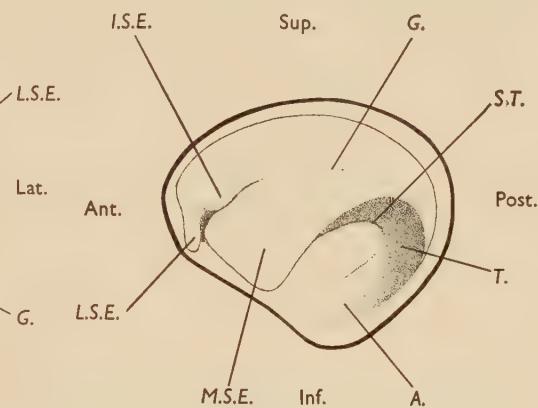


Fig. 4

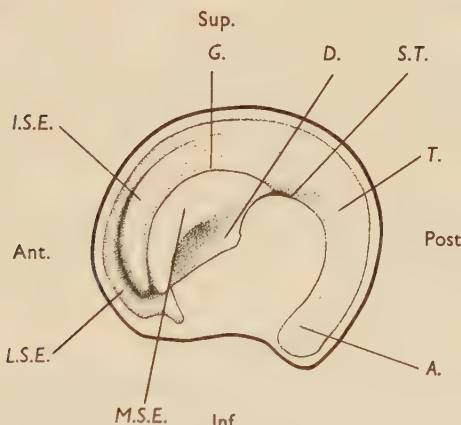


Fig. 5

Fig. 3. Reconstruction of the floor of the left cerebral hemisphere of a 35 mm. c.r. length human embryo, viewed from above. $\times 14$.

Fig. 4. Oblique view, from above and medially, of the interior of a right cerebral hemisphere in which the medial wall and roof have been removed. From a foetus of 65 mm. c.r. length. $\times 5$.

Fig. 5. Medial view of the interior of a cerebral hemisphere in which the medial wall and roof have been removed. From a foetus of 110 mm. c.r. length. $\times 3$.

In the 45 mm. embryo the tail of the caudate nucleus was a broad band, only slightly narrower than the head, which curved round into the temporal pole where it expanded to form the primordium of the amygdaloid nucleus. The development of these structures was completed in the 230 mm. foetus. Prior to this the surface

of the tail had become rounder and more elevated so that it was separated from the side wall of the hemisphere by a deep groove. In its final stages, however, it became depressed into the floor of the ventricle and narrowed relative to the broad head. The majority of these structures were undoubtedly derived from the backward extension of the lateral elevation, and to some extent this was confirmed by the increasing inclination of the posterior end of the groove in the head over to the medial side of the tail. The intermediate component of the head contributed little or nothing to the tail, but the degree of involvement of the medial part was more

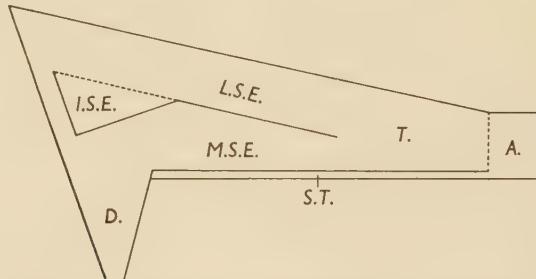


Fig. 6. Diagram to illustrate the development of the human caudate and amygdaloid nuclei. (The dotted lines separate the intermediate striatal elevation and amygdaloid nucleus from the parts from which they were derived.)

difficult to assess. In foetuses over 65 mm. long the posterior end of this elevation extended as a thin sharp band along the tail to the amygdaloid nucleus. When followed forwards this band was continuous with the extension through the interventricular foramen and later provided the path for the stria terminalis. At first this was on the under aspect of the tail, but finally it lay along the medial border of this, when the thalamus had enlarged and filled up the cleft between itself and the tail. It appeared, therefore, that the tail included some of the medial elevation although the amount may have been no more than the strand which had been dragged back.

DISCUSSION

These results, which are summarized in Fig. 6, demonstrated that the head of the caudate nucleus developed from three parts. The first lay in the side wall of the cerebral vesicle and was connected to its fellow across the floor of the diencephalon. Lateral to this, and overtaking it in length and volume, a second part appeared which corresponds with the accounts of Baxter (1953), Frazer (1931) and Hines (1921, 1922). Finally, a third component was introduced which wedged itself between the others, and overgrew the second. The tail and amygdaloid nucleus resulted from the union of the posterior ends of the first two parts forming the head, the greater contributor being the second. The involvement of the primary mass was problematical, but it appeared at least to provide the pathway for the stria terminalis. The nuclei were not fully formed until the completion of foetal life.

The connotation used by authors for the developing parts of these nuclei is confusing because some correlate them with their phylogeny, others relate them to

their form and position and another group use a mixture of both. Until their phylogenetic and functional relationships are correctly established it would seem better to use terms relating to their position and it is suggested they are called medial, intermediate and lateral striatal elevations.

The functional significance of these parts contributing to the caudate nucleus is a matter for conjecture. The suggestion has been made that the medial part is olfactory in function because it appears first. The possibility that the lateral elevation is functionally related to the cerebral cortex is supported, perhaps, by the contention of Elliot Smith (1919) who called it the hypopallium, and said it originated from cortex inrolled around the lateral striate artery. The significance of the intermediate elevation is obscure, but it may be linked with the thalamus because both structures are undergoing their maximal development at about the same time. However, although these speculations are interesting, much more information about these structures will be needed before their functions can be understood.

SUMMARY

1. The surface form of the developing human caudate and amygdaloid nuclei is described.
2. The head of the caudate nucleus is formed by the successive appearance of three parts, termed medial, lateral and intermediate striatal elevations.
3. The tail of the caudate nucleus appears to be principally derived from the second or lateral striatal elevation and the amygdaloid nucleus develops at its posterior end.
4. The contribution of the second or medial striatal elevation to the tail of the caudate nucleus is uncertain, but it appears to provide the pathway for the stria terminalis.

My thanks are due to Prof. D. V. Davies for his encouragement and advice, and also to Dr R. R. Wilson of Paddington General Hospital for provision of some of the material used. I also wish to thank Mr J. S. Fenton and Mr A. L. Wooding for carrying out the photography.

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ABBREVIATIONS

A. Amygdaloid nucleus.
D. Extension of medial striatal elevation into the diencephalon.
G. Groove between the medial and lateral striatal elevations.
I.S.E. Intermediate striatal elevation.
L.S.E. Lateral striatal elevation.
M.S.E. Medial striatal elevation.
P.B. Paraterminal body.
S.T. Strand of medial striatal elevation forming the stria terminalis.
T. Tail of the caudate nucleus.
Th. Thalamus.

GROWTH CHANGES IN SENSORY NERVE FIBRE AGGREGATES UNDERGOING REMYELINATION

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Regeneration studies of the peripheral nervous system have long intrigued the biologist and clinical neurologist alike. Interest in this field is reflected by the copious literature dealing with the qualitative aspects of this type of regrowth which has been appearing for more than half a century. Quantitative data are, however, still comparatively sparse and it was not until Gutmann, Guttmann, Medawar & Young (1942) showed that crushing of a nerve trunk with a smooth tipped forceps caused Wallerian degeneration distal to the site of the trauma without disorganization of the endoneurial tubes surrounding the degenerating fibres that it was possible to eliminate many of the variables inherent in earlier research in this field. Thus the cutting of nerve trunks to produce degeneration of the fibres they contained became unnecessary, and the uncertain but profound effects of the subsequent scar formation and of the probable malaposition of the cut nerve ends no longer required consideration.

That there is considerable normal individual variation within myelinated nerve fibre populations of similar nerve trunks with regard to total numbers and diameter size-frequency distributions has been frequently acknowledged (McCrady, 1934; Gutmann & Sanders, 1943; Aitken, Sharman & Young, 1947; Causey, 1948; Fernand & Young, 1951; Campbell & Murtagh, 1951; Evans & Daly, 1953; Quilliam, 1955b; Graf & Hjelmquist, 1955; Birren & Wall, 1956; Britton & Tomasch, 1956). Since these parameters are the only criteria by which the progress of generalized remyelination can be judged accurately, it is somewhat surprising that such scant attention has been paid to their normal limits of variation in much of the earlier work in this field.

In the present work myelinated fibres in regenerating sural nerve trunks are examined. These nerves are particularly suitable for quantitative study since they possess an unbranched length of about 7 cm. (Quilliam, 1950). Also their peripheral distribution is entirely cutaneous so the findings form an interesting contrast to those of other workers in the field who have used nerves to muscles.

It can now be deduced that the regenerating myelinated fibres of the sural nerve give off no myelinated collaterals. In addition, it has been found that, distal to the lesion, they not only fail to achieve the diameter they exhibit proximally but they also show a centrifugally directed taper which is most marked in the early stages of regrowth. This latter feature is absent both in intact nerves and in regenerating myelinated fibres located central to a lesion. The direction and speed with which the 'wave front' of remyelination spreads centrifugally along the nerve trunk has also been determined.

METHODS

Rabbits, without regard to sex or breed (type), whose body weights exceeded 2.5 kg. were used throughout this study.

Under Nembutal and open ether anaesthesia, the sural nerve was exposed *in situ* at the point at which it left the sciatic trunk to diverge posteriorly. This was usually at a level about 3 cm. distal to the great trochanter of the femur. The nerve was then crushed for 10 sec. using a watch-maker type forceps with smooth blades about 1 mm. wide. The wound was closed and the animal allowed to survive for from 25 to 300 days before the biopsy was undertaken. During this latter procedure the crush site could be identified in early cases by a slight swelling locally and an unusual vascularity of the nerve trunk but, in later cases, no such naked eye differentiation was possible. The nerve was dissected free from the sciatic trunk as far proximally as possible (i.e. from 1 to 3 cm. centripetally), and it was then isolated distally from surrounding tissues until it became macroscopically branched (usually over the distal portion of the posterior aspect of the belly of the gastrocnemius muscle). The complete nerve was then removed from the animal, cut into two or three sections of convenient length, and these were gently stretched over separate oblong cardboard frames to the short sides of which their ends were made to adhere by gentle pressure. Without delay the specimens were placed in Flemming's solution (1% chromic acid 15 ml., 2% osmic acid 4 ml., and glacial acetic acid 1 drop) and allowed to fix for 24–48 hr. Dehydration in ascending concentrations of alcohol was then carried out followed by paraffin embedding. Sample transverse sections 7μ thick were cut at successive 2 mm. levels along each specimen, and these were stained by the Wolter modification of the Weigert-Pal technique (Romeis, 1937). Microphotography ($\times 750$) of suitable sections was then carried out and the negative photographic images of the fibres counted and placed in arbitrary diameter size groups in a similar manner to that fully described in a previous paper (Kitchell, Campbell, Quilliam & Larson, 1955).

Every effort was made to standardize all procedures including the after-care of the animals, in order that strict comparisons could be made between the data obtained from specimens regenerated for slightly different periods of time.

Histograms were constructed from the data obtained and the total cross-sectional area of all the myelinated fibres in every section was calculated in each specimen.

In twelve rabbits, the sural nerves were crushed bilaterally, but only in eight animals were both right and left nerves found to be suitable for quantitative treatment. In the remaining four cases the nerve from one or other side alone proved sufficiently well stained to be subjected to quantification. In a further four animals the sural nerve was crushed unilaterally and the uncrushed contralateral nerves from these animals acted as a control series.

On checking the absolute magnifications of the various sets of microphotographs, it was found that these varied from $\times 750$ to $\times 770$. Thus measurements have been made on some of those whose enlargement exceeded the standard magnification factor by not more than 2.6%, but it was thought unlikely that this error affected the derived data in any important manner.

RESULTS

Proximal to the level of a crush site

Branching with the production of myelinated collateral fibres does not take place. Thus, in each one of the fifteen specimens in which sections at two or more levels in the central segment can be examined, the total numbers of myelinated fibres remain virtually constant and fall within the normal range (Quilliam, 1956). Any differences occurring from level to level are very small and of an order likely to be associated with the experimental error of the methods used. They are, for example, considerably less than those found by Birren & Wall (1956) using different techniques. Thus in specimen no. 35, which has regenerated for 45 days (see Table 1),

Table 1. *The myelinated fibre diameter size-frequency distribution in a sural nerve regenerated for 45 days (specimen no. 35)*

Section level (mm.)	Numbers of fibres in each diameter size group (μ)								Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16		
0	115	787	350	251	156	119	32	5	1815	48,550
6	116	612	402	380	171	112	17	5	1815	51,581
12	151	441	560	355	173	113	18	5	1816	52,989
18	111	564	473	304	230	92	23	5	1802	51,979
22	105	534	527	321	195	90	30	0	1802	52,623
24	69	467	599	372	189	81	24	2	1803	52,693
25	←----- Level of crush site -----→									
26	217	471	657	127	0	0	0	0	1472	23,831
28	157	523	608	169	0	0	0	0	1457	22,262
30	189	350	659	236	0	0	0	0	1434	24,644
32	186	378	579	243	0	0	0	0	1386	23,538
36	276	531	432	98	0	0	0	0	1337	16,222
42	283	369	425	193	0	0	0	0	1270	18,603
46	276	499	360	63	0	0	0	0	1198	13,237
56	127	297	465	188	0	0	0	0	1077	18,564
60	198	496	276	14	0	0	0	0	984	10,232

(Distal end)

the total number of myelinated fibres in the most proximal section available for study (i.e. level 0 mm.) is 1815, whilst that in the section located immediately central to the level of the crush site (i.e. level 24 mm.) it is 1803. At four intermediate levels (i.e. 6, 12, 18 and 22 mm.) the totals are 1815, 1816, 1802 and 1802 respectively. Comparison of the myelinated fibre diameter size-frequency distributions obtained from sections at these levels clearly shows that systematic tapering does not occur. This is confirmed by the study of the total cross-sectional areas of the myelinated fibres as calculated from these same frequency distributions (Quilliam, 1954).

Although the absolute values of the data obtained from the myelinated fibre populations, found in the proximal segments of the regenerating nerves, all fall within the limits prescribed in the earlier series of intact nerves previously referred to, yet there are occasionally, in certain specimens of the present series, differences from level to level between the numbers of fibres allocated to particular diameter size groups which exceed those variations encountered in similar data from the individual specimens of the previous series. Also in several specimens of the present series a few fibres appear at one level only in a diameter size group higher than that

containing the largest fibres in the remaining sections studied from the same segment of nerve (see Tables 2 and 3). Since these comparatively minor discrepancies are randomly distributed, it is probable that they are indicative only of an exaggeration

Table 2. *The myelinated fibre diameter size-frequency distribution in a sural nerve regenerated for 35 days (specimen no. 32)*

Section level (mm.)	Numbers of fibres in each diameter size group (μ)								Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16		
0	43	285	447	244	232	135	38	0	1424	52,864
4	46	256	488	238	226	118	82	0	1454	66,840
6	85	242	439	272	182	104	66	36	1426	57,449
7	←----- Level of the crush site -----→									
8	87	382	637	164	11	0	0	0	1281	22,287
10	182	515	411	41	6	0	0	0	1155	13,814
14	147	434	409	79	0	0	0	0	1069	14,381
16	161	380	336	18	0	0	0	0	895	10,103
20	299	356	213	0	0	0	0	0	868	6,934
22	258	447	110	0	0	0	0	0	815	5,561
30	143	225	246	9	0	0	0	0	623	6,879
34	133	239	160	0	0	0	0	0	532	4,935
36	121	198	132	0	0	0	0	0	451	4,237

(Distal end)

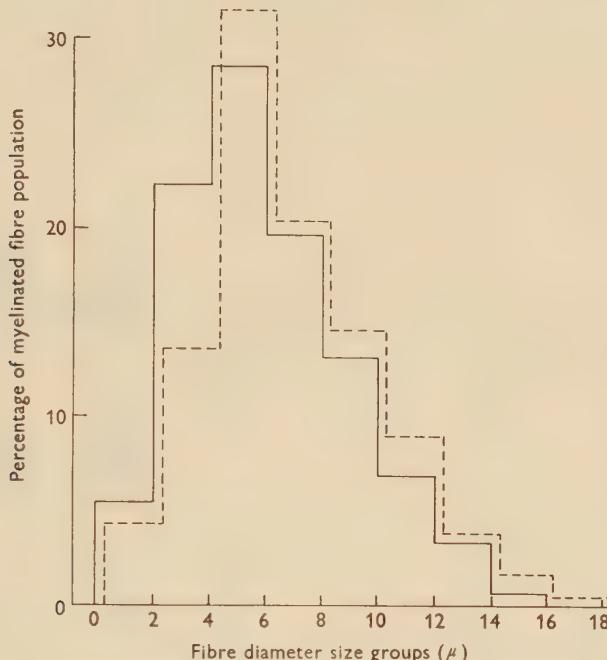
Table 3. *The myelinated fibre diameter size-frequency distribution in a sural nerve regenerated for 65 days (specimen no. 39)*

Section level (mm.)	Numbers of fibres in each diameter size group (μ)								Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16		
0	105	376	401	248	221	111	57	5	1524	53,216
10	79	316	492	263	250	114	10	0	1524	50,137
20	79	340	454	295	199	132	23	0	1522	50,990
22	53	348	513	309	182	96	13	0	1514	46,020
24	30	175	631	419	243	10	0	0	1508	46,185
25	←----- Level of the crush site -----→									
26	194	422	478	356	70	0	0	0	1520	30,675
28	133	410	497	424	40	0	0	0	1504	31,387
30	35	295	641	403	117	0	0	0	1491	37,651
38	80	344	586	342	126	13	1	0	1492	35,387
44	69	250	540	450	179	0	0	0	1488	40,694
48	73	391	586	345	79	1	0	0	1475	31,715
56	126	511	456	302	75	0	0	0	1470	28,945
58	53	477	534	352	35	5	0	0	1456	29,907
60	41	333	641	377	57	4	0	0	1453	32,891
64	51	515	480	326	82	1	0	0	1455	30,236
66	51	394	573	378	49	2	0	0	1447	31,644
68	192	414	456	325	61	0	0	0	1448	28,228

(Distal end)

of the comparatively small irregularities of outline normally found, locally, in intact nerves (Eccles & Sherrington, 1930; Barnes, 1932; Duncan, 1934; Speidel, 1935; Hursh, 1939; Hess & Young, 1952; Quilliam & Sato, 1955). The techniques used did not allow of identification of the precise site or the cause of these irregularities, but it was believed likely that they may have been caused by the perinodal retraction of myelin as described by Sanders (1948) in regenerating nerves, and Causey & Palmer (1952) during the early stages of nerve degeneration.

It seemed possible that the data already to hand might, if suitably arranged, clarify the position with regard to the changes in total (axon and myelin) diameter which, it has been claimed, occur in fibres lying proximal to a lesion during certain stages of regeneration (Sanders, 1948; Weiss & Hiscoe, 1948). Consequently an attempt was made by trial and error to divide the twenty-three specimens available for study into an 'early' set and a 'late' set so that the maximum differences between the pooled data from the two sets might be most apparent. It was found that this



Text-fig. 1. Offset percentage histograms showing the differences between the diameter size-frequency distributions of the myelinated fibres lying proximal to crush sites in two series of sural nerves regenerated for from 25 to 65 days ('early' set—continuous line) in one case and from 75 to 300 days ('late' set—discontinuous line) in the other. (Pooled data have been used, see text.)

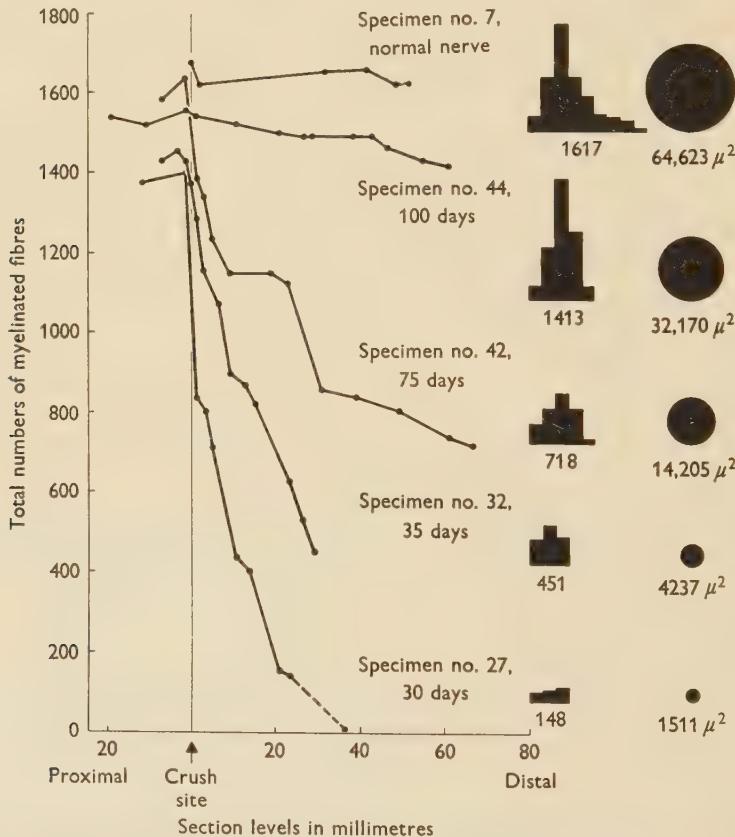
result was best achieved if the 'early' set was formed from the fifteen nerves which had regenerated for from 25 to 65 days and the 'late' set from the eight nerves which had regenerated for from 75 to 300 days (see Text-fig. 1). Although the modal diameter remains in the 4–6 μ size group in both sets, the proportion of fibres exceeding 6 μ in diameter is 45% in the 'early' set, whereas in the 'late' set it is 50%. This proportional discrepancy is more emphasized when the fibre representation in the higher supramodal diameter size groups is examined. These results, together with those appearing below, and also those of the control series are referred to again in the discussion section of this paper as a number of interesting points arise when their interpretation is attempted.

When the pooled data from the four unilaterally crushed sural nerves are compared with those from the whole series of bilaterally crushed nerves, it is found that

the mode of the fibre diameter size-frequency distributions lies in the same group (i.e. $4\text{--}6\mu$) in both cases, and there is very little difference between the proportions of fibres exceeding 6μ in diameter (40% as against 42% respectively). However, the proportions of fibres occupying the upper size groups are slightly different.

Distal to the level of a crush site

After a delay of at least 15 days the process of remyelination proceeds centrifugally along the regrowing fibres starting from the level of the lesion. As regrowth continues, the proportion of fibres which become remyelinated increases, but in spite of a continued gain in diameter few, if any, of the larger fibres achieve their original calibre. At first they all exhibit a marked distally directed taper, but this becomes less pronounced with time (Quilliam, 1955a). No myelinated collateral fibres are formed.



Text-fig. 2. The total number of myelinated fibres at different levels in sural nerves regenerated for various periods of time. (Histograms and circles refer to the most distal sections in each case.)

In the early stages of regeneration (see Tables 1 and 2) more fibres are always counted proximal to the lesion than immediately distal to it, and in the later stages (see Tables 3 and 4), even after equality between total numbers at these two levels

is reached, a progressive diminution in numbers is recorded at levels lying successively more distal to the lesion (see Text-figure 2). This is because at these levels an increasing proportion of the regrowing fibre population comes to possess a diameter of less than 2μ and thus loses its optically demonstrable myelin sheath.

Table 4. *The myelinated fibre diameter size-frequency distribution in a sural nerve regenerated for 300 days (specimen no. 46)*

Section level (mm.)	Numbers of fibres in each diameter size group (μ)										Total number	Total area (μ^2)
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20		
0	102	278	482	277	297	140	118	72	13	4	1783	88,332
10	107	166	477	358	229	230	114	105	31	2	1819	102,117
11	←-----	Level of crush site -----→										
14	71	263	571	405	388	141	58	3	1	0	1851	72,071
18	87	254	656	444	361	67	1	0	0	0	1870	61,721
30	194	249	569	390	319	119	9	0	0	0	1849	60,561
44	68	244	607	365	386	108	6	0	0	0	1784	68,010
(Distal end)												

Table 5. *The myelinated fibre diameter size-frequency distribution in sections taken immediately distal to a crush site in typical sural nerves regenerated for various periods of time*

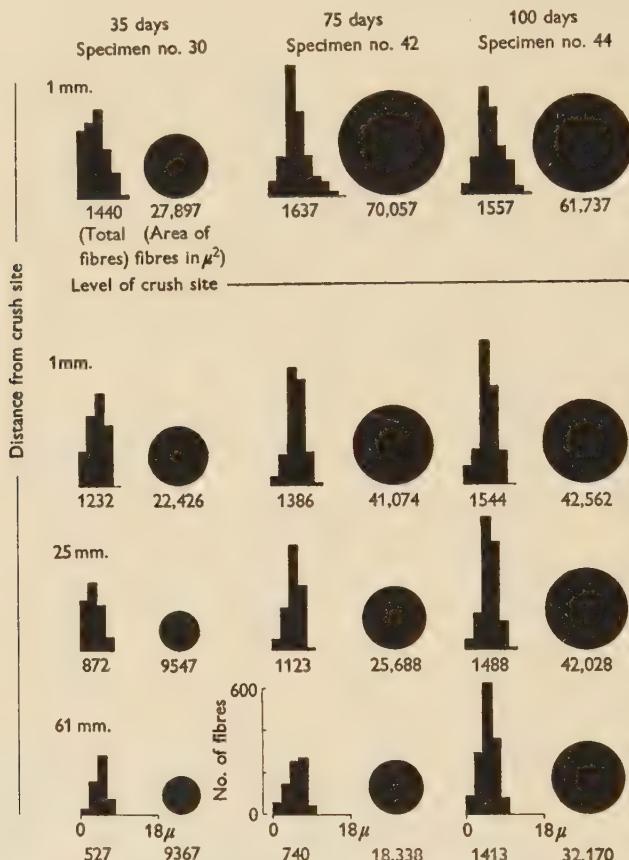
Days re-generated	Numbers of fibres in each diameter size group (μ)									Total no. of fibres	% of prox. total	Total area (μ^2)	% of prox. total
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18				
4	25	102	214	265	74	0	0	0	0	655	42.9	9,644	17.4
7	30	69	310	373	89	0	0	0	0	841	60.2	12,994	23.1
12	35	87	382	637	164	11	0	0	0	1281	89.8	22,287	38.8
3	55	122	301	856	469	64	0	0	0	1812	98.6	41,152	65.1
9	75	38	242	536	505	101	0	0	0	1422	90.1	38,125	61.7
10	100	84	166	672	459	160	3	0	0	1544	99.2	42,562	68.9
3	300	71	263	571	405	338	141	58	3	1851	101.8	72,071	70.6

Table 6. *The myelinated fibre diameter size-frequency distribution in sections taken 3 cm. distal to a crush site in typical sural nerves regenerated for various periods of time*

Days re-generated	Numbers of fibres in each diameter size group (μ)							Total no. of fibres	% of prox. total	Total area (μ^2)	% of prox. total
	0-2	2-4	4-6	6-8	8-10	10-12	12-14				
25	0	0	0	0	0	0	0	Nil	—	Nil	0
30	0	0	0	0	0	0	0	Nil	—	Nil	—
35	121	198	132	0	0	0	0	451	31.6	4,237	7.4
55	95	215	398	336	1	0	0	1045	56.9	22,404	35.4
75	154	264	503	367	23	0	0	1311	82.7	27,356	44.3
100	173	243	634	389	49	0	0	1488	95.6	32,461	52.6
300	68	244	607	365	386	108	6	1784	98.1	63,010	61.7

Tables 5 and 6 summarize these points in selected cases. Only 42.9% of the myelinated fibres present in the proximal segment are found in a section taken immediately distal to the crush site in a typical specimen allowed to regenerate for 25 days. This proportion increases to 60.2, 89.8, 98.6, 90.1, 99.2 and 101.8% in sections at corresponding levels in other typical specimens, which have regenerated for 30, 35, 55, 75, 100 and 300 days respectively. The fact that in the last-mentioned specimen there are 1.8% more fibres distally than proximally does not necessarily indicate that fibre branching has taken place since this small excess falls well

within the limits previously determined for the experimental error of the methods employed (Quilliam, 1956), and similar occurrences are not noted at any other level in the same nerve or at any level in any other specimen.

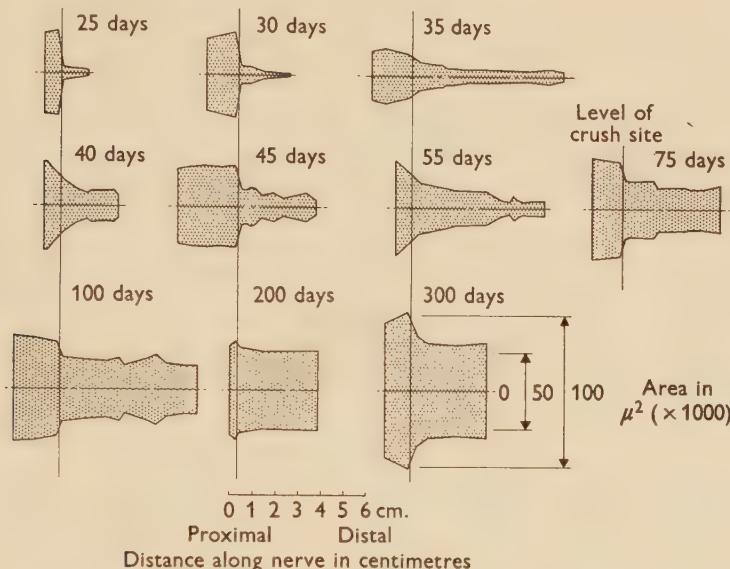


Text-fig. 3. The progressive pattern of remyelination graphically depicted in sural nerves regenerated for 35, 75 and 100 days. (Histograms represent diameter size-frequency distributions and solid circles the total cross-sectional areas of all the myelinated fibres at the various levels indicated.)

When more distal segments of the same regenerating nerves come to be examined it is found that in specimens which have regenerated for 25 and 30 days, no myelinated fibres can be traced in sections taken more than about 2 cm. distal to the crush. In the remaining nerves mentioned above, 31·6, 56·9, 82·7, 95·6 and 98·1 %, respectively, of the fibres found in the proximal segment are present in sections lying 3 cm. distal to the crush site in each case. Text-fig. 3 shows this trend in a graphical form at different levels in three representative specimens regenerated for 35, 75 and 100 days.

The gradual fibre enlargement as regrowth proceeds is well exemplified by the increase in the number of the largest fibres found at corresponding levels in a series of specimens regenerated for progressively longer periods of time. Thus those present

in sections lying immediately distal to the crush site in specimens regenerated for 25 and 30 days lie in the $6-8\mu$ group (which contains 74 and 89 fibres respectively), in the $8-10\mu$ group (which contains 11, 64 and 101 fibres respectively) in specimens regenerated for 35, 55 and 75 days, in the $10-12\mu$ group (which contains 3 fibres) in the specimen regenerated for 100 days, and in the $16-18\mu$ group (which contains 1 fibre) in the specimen regenerated for 300 days (see Table 5).



Text-fig. 4. The total cross-sectional area of the myelinated fibres at various levels in sural nerves regenerated for from 25 to 300 days, represented diagrammatically.

That centrifugally directed tapering of the regenerating fibres occurs can be demonstrated by comparing the size or the number (or both) of the myelinated fibres lying in the supra-modal diameter groups at different levels in each individual specimen of this same series. Thus there are always more fibres of a greater diameter near the crush site than further distally, as can be confirmed by comparing the data just quoted with those which follow. In the specimen regenerated for 35 days (see Table 6) the largest fibres in sections situated 3 cm. distal to the crush site lie in the $4-6\mu$ size group which contains 132 fibres (myelinated fibres have not yet appeared at this level in specimens regenerated for 25 and 30 days). In the specimens regenerated for 55, 75 and 100 days the largest fibres lie in the $8-10\mu$ size group (which contain 1, 23, and 49 fibres respectively) and in the $12-14\mu$ group (which contains 6 fibres) in the specimen regenerated for 300 days.

These effects can be confirmed by comparing the total cross-sectional areas of the fibres at various levels distal to a crush site in individual members of the same series. This is shown schematically in Text-fig. 4.

With reference to the specific examples already used (see Table 5) it can be seen that in a section taken immediately distal to the lesion in the specimen which had regenerated for 25 days, the total cross-sectional area of the myelinated fibres is

only 17.4% of that of the fibres in the proximal segment. At corresponding levels in the other specimens just considered, it can be observed that the figures are 23.1, 38.8, 65.1, 61.7, 68.9 and 70.6% after 30, 35, 55, 75, 100 and 300 days' regeneration, respectively. Since myelinated fibres had not yet reached a level 3 cm. distal to the crush site in the specimens which had regenerated for 25 and 30 days (respectively) no figures can be quoted; but in corresponding sections from the same specimens regenerated for 35, 55, 75, 100 and 300 days the total cross-sectional areas of the myelinated fibres are only 7.4, 35.4, 44.8, 52.6 and 61.7% of that in their respective proximal segments (see Table 6).

Table 7. *Individual variation in the total number and the diameter size-frequency distribution of the myelinated fibres located immediately distal to and also one centimetre distal to the lesion in four different sural nerves regenerated for 35 days after a crush*

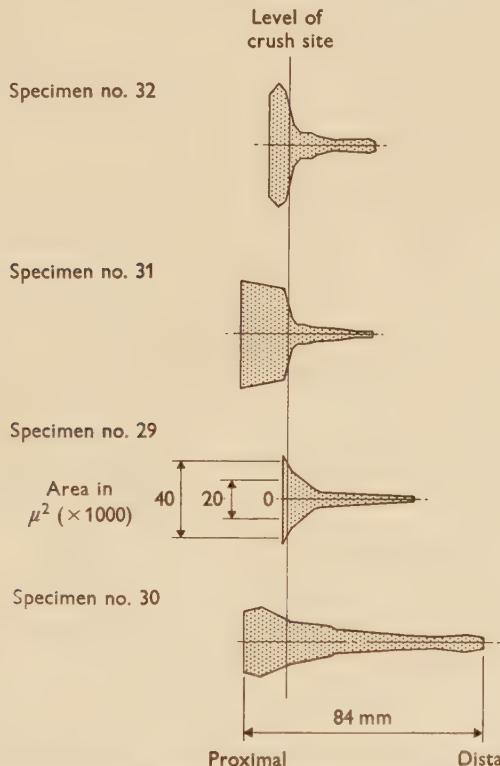
Spec. no.	No. of fibres	% of prox. total	Largest size group (μ)	No. of fibres	Total area (μ^2)	% of prox. area
Level: immediately distal to a crush site						
29	1316	84.2	12-14	4	29,402	61.9
30	1232	85.6	8-10	4	22,426	80.4
31	1180	82.2	6-8	81	15,859	32.5
32	1281	89.8	8-10	11	22,287	38.8
Level: 1 cm. distal to a crush site						
29	669	42.8	6-8	42	9,635	20.3
30	1176	81.7	6-8	82	16,242	58.2
31	766	53.4	6-8	4	7,488	15.8
32	895	62.8	6-8	18	10,103	17.6

The general orderly pattern of progressive remyelination is not masked by individual variation (Quilliam, 1955b). Data were obtained from three nerves regenerated for 25 days, two nerves for 30 days, four nerves for 35 days, two nerves for 40 days and two nerves for 75 days. The apparent discrepancies between the results obtained from individuals in any one of these small subseries are somewhat greater than those occurring between members of a previous series of intact sural nerves. As the results from the subseries of nerves regenerated for 35 days are typical of the remainder, these alone are shown in Table 7 and Text-fig. 5.

The 'wave front' of remyelination travels peripherally from the lesion at a mean rate of nearly 3 mm. per day. Thus myelinated fibres exceeding 2μ in total diameter can be definitely identified in sections lying as far as 14 mm. (mean value of three specimens) from the level of a crush site after 25 days' regeneration whilst in four specimens regenerated for 35 days the comparable mean distance is 43 mm. The process of remyelination has therefore travelled very nearly 30 mm. in 10 days. However, it seems likely that this estimate is low rather than high since when the total numbers of myelinated fibres contained in the most distal sections available come to be considered, it is found that in the earlier (25-day) subseries the mean value is 255, whilst in the later (35-day) subseries it is 426. Evidently, had sections been available in the later subseries in which the fibres were numerically comparable to those counted in the earlier subseries, they would have been located considerably

further peripherally than were those actually studied and the absolute rate of regeneration correspondingly increased.

Qualitative examination of material regenerated for 15 days shows the presence of myelination in a small number of regenerating fibres near the crush site, but none further peripherally. This is slightly earlier than was found in the case of the regenerating laryngeal branch of the recurrent laryngeal nerve (Evans & Murray,



Text-fig. 5. The total cross-sectional area of the myelinated fibres at various levels in four sural nerves each regenerated for 35 days after a crush, represented diagrammatically.

1956). Since Gutmann *et al.* (1942) found an average delay of 5·2 days before unmyelinated regenerating axon tips could be found distal to a crush lesion, a further 10 days at least is necessary for these small axon tips to enlarge to a diameter size sufficient to attract a myelin sheath thick enough to be rendered optically visible by routine myelin stains. In turn, this implies that between the 15th and 25th days of regeneration, the rate of distally directed advance of the process of remyelination is somewhat slower than after the 25th day—having taken 10 days to traverse a mean distance of only 14 mm. The argument already stated that this type of data leads to an underestimate rather than an overestimate is even more applicable in this particular situation than in the one obtaining after 25 days' regeneration.

The weight, sex or breed (type) of animal does not materially influence the rate of remyelination in a given specimen.

DISCUSSION

(1) *Changes in the myelinated fibres located central to the lesion*

It is evident that the myelinated fibres lying proximal to a crush site do not change greatly in total diameter as regeneration proceeds, yet the differences which do exist between the fibre diameter size-frequency distributions derived from the 'early' and the 'late' sets of pooled data, although small, are significant at the 5% level of probability. This does not necessarily mean that these real differences are attributable solely to the regeneration processes since they could also be brought about by some other concurrent circumstance. Thus since the 'late' set of animals was allowed to survive for a longer mean interval than the 'early' set, it seems entirely possible—indeed likely—that the differences between the data from the two sources are due to the operation of an associated ageing process.

Similar considerations suggest that the differences which exist between the myelinated fibre diameter size-frequency distributions of the pooled data obtained from the set of bilaterally crushed and the set of unilaterally crushed sural nerves are also due to differing survival periods, i.e. from 25 to 300 days in the former set and from 45 to 65 days in the latter set. That this is likely is supported by the results of a more restricted comparison in which data are only used from those members of the bilaterally crushed set whose survival periods correspond most closely to those of the members of the unilaterally crushed set. It is then found that the most marked of the discrepancies (i.e. those which relate to the numbers of fibres located in the higher diameter size groups) almost completely disappear.

(2) *The effect of a contralateral operation*

The intact nerves from the four unilaterally operated animals provide an interesting opportunity to make a comparison with the eleven normal nerves from a previous series of unoperated animals and to check the assertion by Greenman (1913) that a unilateral crush operation affects the contralateral intact nerve. In the present series, the mode of the pooled fibre diameter size-frequency distributions in both cases lies in the same group (i.e. 4–6 μ), but in the intact specimens from the unilaterally operated animals 49% of the myelinated fibres exceed 6 μ , whilst in the intact specimens from the unoperated series of animals, this figure is only 43%. Again statistical analysis suggests that this small difference is significant, but in view of the fact that the unilaterally operated rabbits remained in the animal house for at least 6 weeks before biopsy and the previously unoperated rabbits were sacrificed within 1 week of admission, it would be presumptuous, without additional evidence, to ascribe the divergences noted solely to the presence of a contralaterally regenerating nerve and it is thought more likely that they are also a reflexion of an ageing process.

(3) *Changes in the myelinated fibres located peripheral to the lesion*

The process of centrifugally directed growth exhibited by the regenerating fibre tips is first detected by the appearance initially of a few and later of many small myelinated fibres adjacent to the crush site. As regrowth continues, an increasing proportion of these fibres reaches successively more distal levels. At first the fibres

taper markedly within the peripheral stump, but later they become almost cylindrical though the larger ones seldom, if ever, achieve their original diameter. Myelinated collateral fibres never appear.

Present concepts of fibre regrowth are inadequate by themselves to explain the differences between this regenerative pattern and the corresponding one described by earlier workers in which muscle or mixed nerves are studied and early profuse myelinated fibre branching noted (Shawe, 1955) and the peripheral fibres are found to regain their original calibre (Gutmann & Sanders, 1943).

Since peripheral factors are unlikely to become effective until the regenerating fibres have made contact with the organ to be re-innervated, and it is difficult to imagine how differing local factors could be produced by the microscopically almost identical structures concerned, it is necessary to assume that the regenerative activity is controlled and the degree of proliferation determined by a central factor—at any rate during the early stages.

(a) *Branching*

It may be that the cell bodies of motor neurons associated with efferent fibres ('axons') of a given diameter normally found in peripheral nerve trunks possess an innate capacity to deliver more replacement axoplasm to their regenerating fibre tips than do the cell bodies in the dorsal root ganglia associated with similarly situated afferent fibres ('dendrites') of the same calibre. It is not clear whether this is simply because of a difference in size or environment between the two kinds of cell bodies or because the former receive frequent physiological stimulae from higher centres even during the early stages of regeneration whilst the latter must await re-innervation and probably even partial maturation of their end organs before a corresponding activity can recommence. However, it is interesting to recall that the actual rate at which fibre tips grow distally is closely similar in both muscle and cutaneous nerves and in young and old animals (Gutmann *et al.* 1942). Basically, all that is involved in each case, locally, is the passage of axoplasm along endoneurial tubes and since the physical properties of these components are likely to be closely similar in the two functionally dissimilar types of nerve fibre, it would appear that the greater volume of replacement axoplasm assumed to be available to the efferent fibres cannot accelerate the rate of forward growth above a maximum determined by common mechanical factors, but that it is expended instead in the formation of numerous stout collaterals.

(b) *Tapering*

Marked tapering occurs in myelinated fibres during the early stages of regeneration of mixed, muscle, and cutaneous nerves (Gutmann & Sanders, 1943; Aitken *et al.* 1947; Quilliam, 1950). This surely demonstrates that the source of the material (axoplasm) which forms the regrowing fibres is from above the lesion. At first, in the present work, fibre elongation via the ready-made pathway of endoneurial tubes of the distal segment is the dominant activity but, even before the fibre tip reaches its end organ and must perforce stop elongating, an increase in diameter is noted. This process of distention lags at the more peripheral levels as can be well seen during the middle stages of regeneration. Later on, the discrepancies between the

diameter sizes of the regenerating fibres from level to level distally become less obvious and are eventually only easily detected in those fibres resident in the higher diameter size groups as few, if any, of these latter fibres regain their original diameter.

It seems doubtful if these facts represent a fundamentally different situation from that obtaining in the fibres of regenerating muscle nerves, although many of their fibres, distally, do seem to achieve their original calibre. It may be that an endoneurial tube shrinks irreversibly if it is left incompletely filled for too long, and thus limits subsequent fibre dilatation (Sanders & Young, 1944). The early and profuse branching of the regenerating fibres in muscle nerves (Shawe, 1955), by occupying more space, may be presumed to be more effective in keeping the endoneurial tubes dilated than is the case in the less prolific fibres of cutaneous nerves.

(c) *Remyelination*

The rate at which the wave front of remyelination spreads along the regrowing fibres of a regenerating nerve trunk has not been measured before. The present work provides proof that it proceeds in a centrifugal direction from a starting-point lying immediately distal to the level of the lesion. It is intriguing to find that the speed with which this process travels is very similar to that of the peripheral advance of regrowing axon tips which precedes it and of 'functional completion' which follows it (Gutmann & Guttmann, 1942; Gutmann *et al.* 1942). It is not easy to explain this away as a coincidence nor will it be possible to support the plausible suggestion that the spread of all three processes is governed by the same physical laws until the visible changes (if any) which accompany 'functional completion' have been identified.

(d) *Unmyelinated fibres*

A more complete picture of the changes which take place in regenerating sural nerves must await reliable quantitative information relating to their non-myelinated fibre content. Shawe (1955), using a modified Bodian staining technique, published figures of the total fibre content (myelinated and unmyelinated) of both sural nerves and nerves to the medial head of the gastrocnemius muscle. However, comparison of these data with those from Weigert preparations in which only myelinated fibres were stained (Quilliam, 1956) fail to demonstrate the presence of a sizeable proportion of non-myelinated fibres in either of these nerves. However, a subsequent personal study (unpublished) using Ranson's pyridine-silver technique has shown that a very substantial number of non-myelinated fibres are, in fact, located within the normal sural nerve, and the results of Shawe's (1955) use of the same stain in the nerve to the medial head of the gastrocnemius muscle suggest a similar situation there as well. Evidently, in the circumstances quoted, the particular modification of the Bodian stain used was not suitable for showing all the non-myelinated fibres present.

SUMMARY

1. During regeneration of the sural (cutaneous) nerve following a crush type of operation, no myelinated collateral fibres are formed.
2. Proximal to the lesion, the myelinated fibres are similar, at all stages, to those

studied in an earlier series of intact sural nerves in so far as total numbers, diameter size-frequency distribution and the absence of tapering are concerned.

3. In the distal segment, optically demonstrable myelin sheaths first appear on a proportion of the regrowing fibres adjacent to the crush site about 15–20 days after the operation. Thereafter, the number and diameter of these myelinated fibres rapidly increase as the period allowed for regrowth becomes successively longer and, at the same time, remyelination can be detected at progressively more distal levels.

4. The 'wave front' of remyelination proceeds centrifugally at a rate of 3 mm. per day between the 25th and 35th days of regeneration.

5. All the remyelinated fibres show a distally directed taper, but this effect becomes less marked as the regeneration period increases. However, few if any of the larger fibres in the peripheral stump ever attain the diameter of their parents centrally.

6. The remyelination performance of regrowing fibres in a nerve on one side of the body is unaffected by regeneration taking place in the corresponding nerve on the other side of the body. The myelinated fibres of unilaterally intact nerves are not influenced by regeneration taking place in their contralateral fellows.

7. Individual variation between data obtained from different specimens allowed to regenerate for identical periods of time is greater than that found between members of the series of intact sural nerves previously studied but does not mask the orderly pattern of progressive remyelination.

I wish to thank Prof. J. Z. Young for his help and encouragement during this study. I am also indebted to Mr D. A. Sholl, B.Sc., for guidance in matters statistical, to the late Mr T. Quilliam, B.Sc., for checking the calculations and to the technical staff of the Department of Anatomy, University College London, for much assistance. Text-fig. 1 was drawn by Miss E. R. Turlington of London, England, and the remainder by Mr James R. Piotrowski of St Paul, Minnesota, U.S.A.

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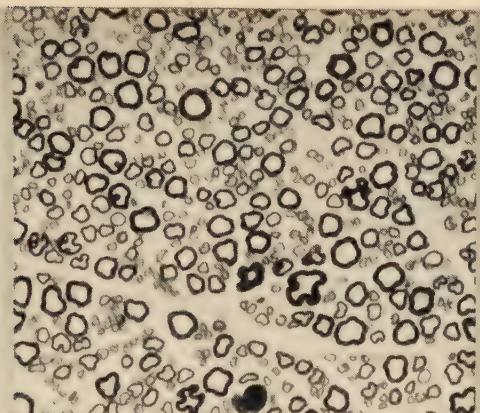
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EXPLANATION OF PLATE

Photomicrographs of transverse sections (7μ thick) taken at various levels in sural nerves of adult rabbits stained by a modified Weigert Pal technique. ($\times 350$.)

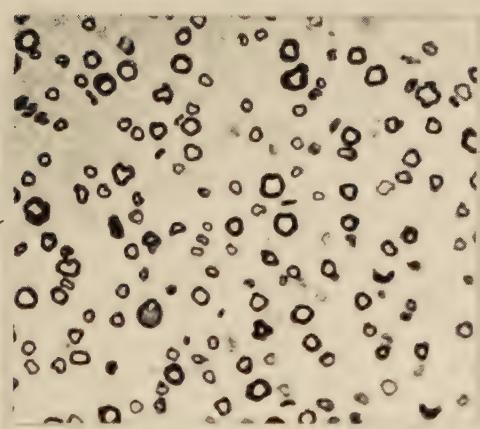
- Fig. 1. From an intact animal (mid thigh).
- Fig. 2. Immediately distal to a crush performed 75 days previously.
- Fig. 3. 60 mm. distal to a crush performed 75 days previously in the same animal.
- Fig. 4. Immediately proximal to a crush performed 25 days previously.
- Fig. 5. Immediately distal to a crush performed 25 days previously in the same animal.
- Fig. 6. 20 mm. distal to a crush performed 25 days previously in the same animal.



1
(Normal)

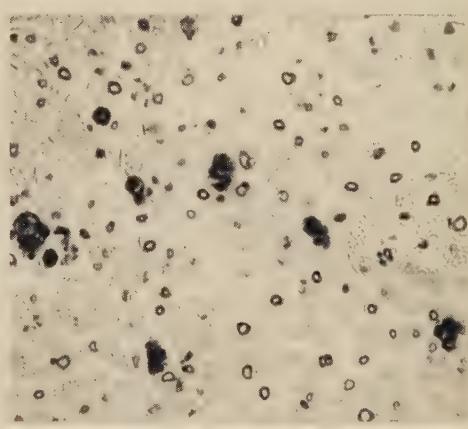


4
(6 mm. proximal)

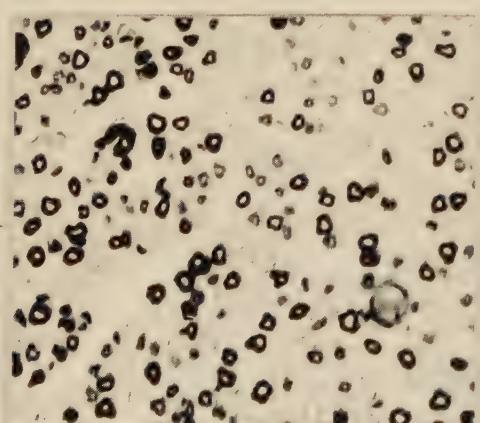


2
(Immediately distal to crush site)

75
days



5



3
(60 mm. distal)



6
(20 mm. distal)

25
days

ELECTRON MICROSCOPIC STUDY OF THE SUPERIOR CERVICAL GANGLION

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INTRODUCTION

Electron microscopic studies of the neurone including both the cell body and its processes have been confined in most instances to the study of normal tissue. The main published work with experimental material has been an analysis of the changes in the neurones of the hypoglossal nucleus after section of the hypoglossal nerve (Hartmann, 1954), the changes in the dorsal root ganglion after section of the peripheral trunk by Causey & Hoffman (1955), and the alteration in the synapse after extirpation of the ventral vestibular ganglion (de Robertis, 1956). In the superior cervical ganglion preliminary data from two ganglia after pre-ganglionic section were reported by Causey & Hoffman (1956a). The present paper extends this investigation to include section of both pre- and post-ganglionic trunks.

The superior cervical ganglion is particularly useful for this type of study in that it is easily isolated and of relatively simple structure. It is possible to alter neuronal activity by removal of part of the cytoplasm by post-ganglionic section and also to study the effect of altering the cell environment by pre-ganglionic section separately or in combination with post-ganglionic section.

In the present study material was fixed with osmium tetroxide, which is generally accepted as providing an acceptable approximation in structure to the living cell provided that the fixation and preparation of the tissue for electron microscopy is carried out with all necessary precautions. Accepting this useful and as far as is known accurate artefact, we have attempted to follow the changes produced by experimental methods in conjunction with a standard preparative technique.

MATERIAL AND METHODS

Pairs of rabbits were anaesthetized using intravenous Nembutal followed by ether. In one series the sympathetic trunk was excised for approximately 1 cm. in the neck, in another the post-ganglionic trunks were cut. Normal and operated ganglia were then removed 2, 3, 7, 14 and 28 days later from the anaesthetized living animal. In a further series 7 days after section of the post-ganglionic fibres the pre-ganglionic trunk was excised, and 7 days later the ganglia were removed.

Each ganglion was cut in two, one half fixed in formol mercuric chloride fixative (Carleton & Leach, 1947), embedded in paraffin wax, and sections 5μ thick stained by Holmes's silver method, Unna's polychrome methylene blue and Galloeyanin (Pearse, 1953). The other half was fixed for 2 hr. in buffered isotonic 1% osmium tetroxide. The material was washed overnight in running tap water and dehydrated in a series of water/ethanol mixtures; finally embedded in a 90% butyl-10% methyl methacrylate mixture with 0.25% benzoyl peroxide added as catalyst; exposed to

ultraviolet light and baked at 56° C. to obtain blocks of reproducible cutting quality. The material was sectioned on a Cook and Perkins microtome using glass knives, and the sections mounted on carbon films, without removing the methacrylate. The electron microscopes used were the Metropolitan Vickers E.M. 4, with a resolution of 100 Å., and the E.M. 6, the latter being a high resolution instrument.

RESULTS

The elements which we have distinguished in the superior cervical ganglion and the pre-ganglionic trunk can be roughly divided into five groups: (1) blood vessels, (2) collagen fibrils, (3) myelinated fibres and bundles of non-myelinated fibres in association with Schwann cells, (4) satellite cells, (5) neurones.

Blood vessels are common throughout the ganglion and it is possible to distinguish two types. Pl. 1, fig. 3, shows the relatively rare vessel with a lumen containing a blood corpuscle. It possesses an endothelial lining, a layer of smooth muscle containing mitochondria and an outer layer consisting of bundles of collagen and cells containing nuclei. To the left of this is a vessel of smaller diameter consisting of a single layer of cells surrounding the lumen.

The spaces between blood vessels, nerve fibres and neurones contain diffuse areas of increased electron density and collections of fibrils which, where cut longitudinally, exhibit banding at 600 Å. intervals characteristic of collagen. Surrounding the ganglion and pre-ganglionic trunk is a capsule consisting of an inner layer of three or four layers of fusiform cells arranged concentrically, and surrounding this is an electron dense layer 5–10 μ thick.

These capsular and vascular structures occur throughout and will not be considered in further detail.

Nerve fibres. At the caudal end of the ganglia the myelinated fibres of the pre-ganglionic trunk split into large groups of non-myelinated nerve fibres and their termination on a neuronal surface is shown in Pl. 2, fig. 4. Here several non-myelinated fibres contained in the satellite cell surrounding a neurone are seen in relationship to one another and to the neuronal surface. Their axoplasm contains mitochondria and vesicles, and is surrounded by a cell wall consisting of two electron dense lines separated by a distance of 200–300 Å. While the inner line is complete, the outer line is continuous with the inner cell layer of the satellite cell wall by means of a mesaxon which consists of two electron dense lines separated by a distance of approximately 200 Å.

All the bundles of non-myelinated nerve fibres between the neurones of the superior cervical ganglion are enclosed in Schwann cells, and where there is a preponderance of cytoplasm it is possible to distinguish their mesaxons. There is no detectable difference between the structure of the satellite cells and Schwann cells and there may be continuity of cytoplasm between them, as in Pl. 1, fig. 1. More frequently the Schwann cells bearing the penultimate fibres which form the axosomatic synapse cluster in layers over the neuronal surface (Pl. 3, fig. 8), and all stages between these extremes are seen.

In shape the nuclei of the satellite cell layer and the Schwann cells are similar. They are round or ellipsoid, occasionally irregular, with a mean diameter of 5 μ

(Pl. 1, figs. 1, 2; Pl. 3, fig. 8). The nuclear membrane is obscured by fine electron dense material aggregated on the inner border in irregular clumps. Some sections reveal, however, that it is composed of two electron dense laminae separated by a distance of 200–300 Å. It can be seen from Pl. 1, fig. 1, that the nucleolus consists of a simple mass of tangled electron dense particles without a limiting membrane. High powers reveal that this is composed of particles approximately 100 Å. in diameter.

In representative sections, such as Pl. 1, figs. 1, 2 and Pl. 3, fig. 8, apart from blood vessels, all supporting cells in the superior cervical ganglion are Schwann cells, or satellite cells, and if fibroblasts are present they are so closely related to Schwann cells as to make it extremely difficult to distinguish the two.

The structure of the neurone. The neurones of the superior cervical ganglion are multi-polar cells abundant at the cephalic end of the ganglion and varying in size within a range 20–70 μ in diameter. They are bound by a cell wall which separates the neuronal from the satellite cytoplasm and appears as two electron dense lines separated by a distance of approximately 300 Å. The neurones contain one to three nuclei, with a mean diameter of 11 μ, which are surrounded by the neuronal cytoplasm which contains mitochondria, granules and vesicles (Pl. 1, figs. 1, 2; Pl. 2, figs. 4–6).

The nucleus. This consists of three main groups of structures—the nucleoplasm, the general appearance of which depends on fixation, the nucleoli and the nuclear membrane. The nucleoplasm consists of two types of particles, the first being dense and elongate, 100 Å. in diameter, and the second is finer and difficult to define. The nucleoplasm appears to be very sensitive to fixation; in well-fixed material the particles are evenly distributed, with occasional concentrations such as in Pl. 2, fig. 6. In inadequately fixed material the particles concentrate together to form a coarse reticulum. The nucleoli are identical in appearance to those described in other cells. There is no limiting membrane and they consist of a tangled mass of dense granules approximately 100 Å. in diameter. The nuclear membrane consists of two electron dense laminae separated by a distance of approximately 300 Å. (Pl. 2, fig. 4). Spaced along this membrane at irregular intervals are areas of increased electron density approximately 1000 Å. in diameter. Seen in surface section these areas are circular with a central boss. They seem to be comparable to the nuclear pores of other authors.

Cytoplasm. It is possible to distinguish three main groups of structures in the neuronal cytoplasm: mitochondria, Nissl substance and vesicles. The mitochondria (Pl. 2, fig. 4) are electron dense structures, 2–4 μ long and 0·2 μ in diameter. Bent and branching forms occur in all normal material. They consist of a limiting wall made up of two electron dense lines with the inner wall invaginated into the body of the mitochondria to form cristae mitochondriales. The Nissl substance consists of granules, 100 Å. in diameter, in agreement with the findings of Palay & Palade (1955). The vesicles seen in neuronal cytoplasm are associated with three structural elements, mitochondria, Nissl substance and Golgi apparatus. The mitochondrial vesicles are within the body of the mitochondrion, which they distend (Pl. 1, fig. 2) in a manner which differs from cell to cell. In appearance these vesicles are round, often bridged by strands of material, and possess an irregular inner margin. The vesicles associated with the Nissl substance are characteristic, without a clearly

defined wall, and are irregular, elongate sinuses, associated with concentrations of electron dense granules. The Golgi apparatus in the superior cervical ganglion consists of tight groups of vesicles near to the nucleus (Pl. 1, fig. 2; Pl. 2, fig. 6). We have not been able to detect any membranes associated with this structure.

Post-ganglionic section. Nuclear changes occurred within 48 hr. of post-ganglionic section (Pl. 5, fig. 15) with marked convolution of the nuclear membrane and loss of contrast between the nucleoplasm and cytoplasm. Seven days after section both the nucleus and cytoplasm are disorganized. Recognizable mitochondria have disappeared and the cytoplasm is full of vesicles with irregular strands of material bridging them similar to mitochondrial vesicles. The nucleoplasm is coarsely reticular and is also filled with vesicles; the nucleolus is swollen and ill defined, and it is difficult to define the nuclear membrane. Under the light microscope the basophilia of the nucleoplasm has increased. The galloxyanin test is strongly positive, indicating that this is probably due to an increase of ribonucleic acid. The bundles of nerve fibres between the neurones are so altered that it is difficult to define them with clarity, and the intercellular material is filled with red cells, debris and vesicles. Single cells with their cytoplasm filled with masses of dense material and the remains of nerve fibres lie amongst this material, and Pl. 3, fig. 10, shows such a cell with an amoeboid margin.

Fourteen days after section the most striking feature is the vast number of nerve fibres which appear between the neurones (Pl. 4, fig. 11), and all stages of the association between these new fibres or sprouts and Schwann cells can be seen. Pl. 4, fig. 12, gives an indication of how the Schwann cell initiates this process, not unlike a cell behaving as a phagocyte, and Pl. 4, fig. 13, at a higher resolution, shows details of the association; here a number of nerve fibres are cut transversely and lie in invaginations of the cell wall, dragging the inner membrane with them to form the beginnings of the mesaxon. The mitochondria in the neuronal cytoplasm are vesicular in many cells and are often associated at one end with the nuclear membrane. Pl. 5, fig. 17, suggests that they are budded off from the nuclear membrane, for here there is a continuity of membranes between a mitochondrion and the nucleus. Attention has also been drawn by Causey & Hoffman (1955) to the association of vesicles, which they consider to be early stages in the formation of mitochondria, and the nuclear membrane in the spinal ganglia.

Twenty-eight days after section the number of nerve fibres between the neurones has diminished and at the same time the number of Schwann cells has decreased. The cytoplasm of the neurone is full of mitochondria distended with vesicles (Pl. 5, fig. 16). A number of the nerve fibres between the neurones are altered in a similar fashion (Pl. 3, fig. 7) and lie among otherwise normal nerve fibres.

Pre-ganglionic section. The early effect of the pre-ganglionic section on the bundles of fibres between the neurones is seen in Pl. 3, fig. 9, which is from a ganglion 3 days after excision of a length of the pre-ganglionic trunk. The number of fibres occurring singly or in small groups seems to have increased, presenting an appearance of large bundles breaking up into the smaller elements. Very little change is apparent in the fine structure of the terminals (Pl. 5, fig. 18). Seven days after section a very profound change identical to that seen after post-ganglionic section has taken place,

and interpretation is difficult. Seven days later, however, it can be seen that in fact the number of terminal nerve fibres surrounding the neurone has diminished and, in view of the fact that 1 cm. of the pre-ganglionic trunk was excised 2 cm. away from the ganglion, these residual fibres can hardly be regenerating pre-ganglionic fibres. The number of nerve fibres between the neurones has not diminished either, and it was felt in view of these findings that the greater part of the interneuronal nerve fibres are in fact post-ganglionic, and that the residual fibres in intimate contact with the neurone are association fibres between nerve cell bodies in the superior cervical ganglion. Twenty-eight days after section the greater part of the neurone is free from satellite cells.

Post- and pre-ganglionic section. In order to determine whether the sprouts formed 14 days after post-ganglionic section were derived from the pre-ganglionic trunk by collateral sprouting, the pre-ganglionic trunk was excised 7 days after post-ganglionic section and the material examined 7 days later. It is apparent from Pl. 5, fig. 14, that the number of new fibres formed has not diminished and is comparable to the ganglion 14 days after post-ganglionic section. At the same time the neuronal cytoplasm is nearly normal in appearance and bears little resemblance to the cell 7 days after pre-ganglionic section alone.

DISCUSSION

The question of fixation in this experimental material is of first importance. All the material has been treated by exactly similar techniques such as to produce adequate fixation in normal material, and it is probable that fixation is also adequate in the later experimental material. For instance in the 28-day material after post-ganglionic section it will be noted that the small fibres between the cells are adequately fixed and that the vesicular appearance of the neuronal cytoplasm is also reproduced in those axonal processes that are cut near the cell body and have axoplasm similar to the cytoplasm of the neurone such as is shown in Pl. 5, fig. 16. On the other hand, 7 days after either pre- or post-ganglionic section the picture obtained precludes adequate interpretation, although the fixation techniques are identical with those for normal material. Similar effects have been seen by Palade (1952) with unbuffered or acid fixative, and further work is in progress to see if this type of change produced by either pre- or post-ganglionic section in the ganglion can be countered by alteration in the pH of the fixative.

It is clear, however, that the difficulties in visualizing neuronal appearances after experimental procedures are due to alterations in the environment resulting from metabolic activity. This is indicated by the mitochondria which show a marked cyclical behaviour, disappearing at 7 days in the form of vesicles, appearing once again at 14 days and disappearing to a rather variable extent at 28 days after pre- and post-ganglionic section. It is known in other tissues that vesiculation of mitochondria can occur under a variety of conditions of altered cell metabolism (Danon, 1952; Dempsey, 1956), cell damage (Rhodin, 1954; and see *S.E.B. Symposium*, 1957, no. 10), and Hartmann (1956) also describes vesiculation of the mitochondria in a neurone in response to an increased level of cortisone known to be produced in excess by operative procedures; this confirms the earlier light microscope observa-

tions of Lowe, Mackinney & Sarkaria (1955). This change, once excited, is not immediately reproducible, since if the post-ganglionic fibres have already been severed there is little change after pre-ganglionic section.

While it is clear how post-ganglionic section produces neuronal damage, it is less clear why pre-ganglionic section also produces the profound changes which are seen under the electron microscope. However, similar observations have been made by Hamlyn (1954) using the light microscope, and the importance of the proper stimulation of cells has also been shown by Brattgard (1952) in the case of the retina in early post-natal rabbits, and more recently by de Robertis & Franchi (1956) in adult animals.

Section of the pre-ganglionic trunk causes the disappearance of many nerve fibres in the satellite cell of the neurone, but does not appear to cause a diminution of fibres between neurones. It is thought that the majority of nerve fibres in the inter-neuronal bundles are processes of neurones within the superior cervical ganglion. This supports the view of de Castro (1932) that 'the absolute lack of axonic collaterals in the autonomic ganglion cell is functionally compensated by the occurrence of numerous association dendrites by which impulses enter into direct relation under the action of a suitable nervous impulse'. The impression gained, without supporting quantitative data, is that 14 days after pre-ganglionic section there is actually an increase in the number of the above fibres, which may be due to collateral sprouting. In this respect it is relevant that Murray & Thompson (1957) have noted that collateral sprouting occurs readily in this ganglion in response to trauma or the presence of adjacent degenerating fibres.

One of the more striking effects of post-ganglionic section is the production of large numbers of sprouts within 14 days of neurectomy. The contrast between the disorganized ganglion at 7 days, with its altered neurones, the debris and red cells, and the integrated appearance of 14-day-old material is very striking. The production of sprouts after neurectomy is, of course, well known in peripheral nerves. Particular interest lies in the fact that all the new fibres are associated with Schwann cells. These are proliferating maximally 14 days after post-ganglionic section, as Abercrombie & Johnson (1946) also observed in peripheral nerve after neurectomy. Further, at 28 days after section there is a decrease in the number of Schwann nuclei with a concomitant decrease in the number of fibres. It seems, therefore, that in nervous tissues proliferation of Schwann cells provides some indication of the degree of sprouting and hence an indication of neuronal activity.

From their structure in the normal ganglion and behaviour in experimental tissue it appears that satellite cells and Schwann cells are identical. The continuity of cytoplasm between satellite and Schwann cells will have important implications in considering the mechanisms of nervous transmission. The presence of Schwann cells acting as macrophages also emphasizes that they can subserve an additional purpose in the ganglion, acting as cells which can clear the debris caused by the reaction of neurectomy. Nageotte (1932) noted that two stages appear to be involved in the formation of collagen in peripheral nerves; one of these forms in close association with the nerve bundles at the expense of 'the primitive membrane of Schwann and the septae which spring from it'. Our results suggest that Schwann cells may be involved in the process of reformation of the collagen fibres also. It would appear

from our results, therefore, that Schwann cells are capable of a multiplicity of functions in response to various conditions imposed on the nervous system.

Material 14 days after post-ganglionic section is interesting in that the formation of mesaxons may be studied in the adult tissue. Our observations are similar to those made by Gasser (1952), Geren (1954) and Causey & Hoffman (1956b). The mesaxon is composed of two dense layers, not four as would be expected if it were formed by a simple invagination of the double cell wall layer of the Schwann cell, and the distance between the membranes of the mesaxon is even less than the distance between the two layers of the cell wall of this cell; not, as would be the case if it were composed of four membranes, twice the thickness of the Schwann cell wall.

Attention has been drawn to the association of mitochondria with the nuclear membrane by Causey & Hoffman (1955), and by Barer & Joseph (1957), and our observations support the view that mitochondria may be formed at or from the nucleus. It is of interest that the diameter of the junction between mitochondrion (noted in Pl. 5, fig. 17) and the nuclear membrane (approximately 1000 Å.) is the same as that of nuclear pores described in the neurone by Dawson, Hossach & Wyburn (1955). The appearance is very similar to the pores formed on yeast cells in the process of budding the daughter cells (Barton, 1950), and it is suggested that the pores on the nuclear membrane could be former sites of mitochondrial formation.

SUMMARY

1. The superior cervical ganglion has been examined in ultra-thin sections under the electron microscope. The changes produced by pre- and post-ganglionic section have been studied.
2. The mitochondria show marked vesiculation or disintegration 7 days after section and reappear as definitive structures by 14 days. The relation of the mitochondria so formed to the nuclear membrane is illustrated and discussed.
3. After pre-ganglionic section it was found that the fibre loss is most marked close to the neurones and that large numbers of post-ganglionic fibres persist between the neurones.
4. Schwann and satellite cells show similar activities when stimulated by section of the nerve trunks, and it has not been possible to distinguish fibroblasts as a separate class.

We wish to thank Mr S. A. Edwards for technical assistance, and the British Empire Cancer Campaign for financial support.

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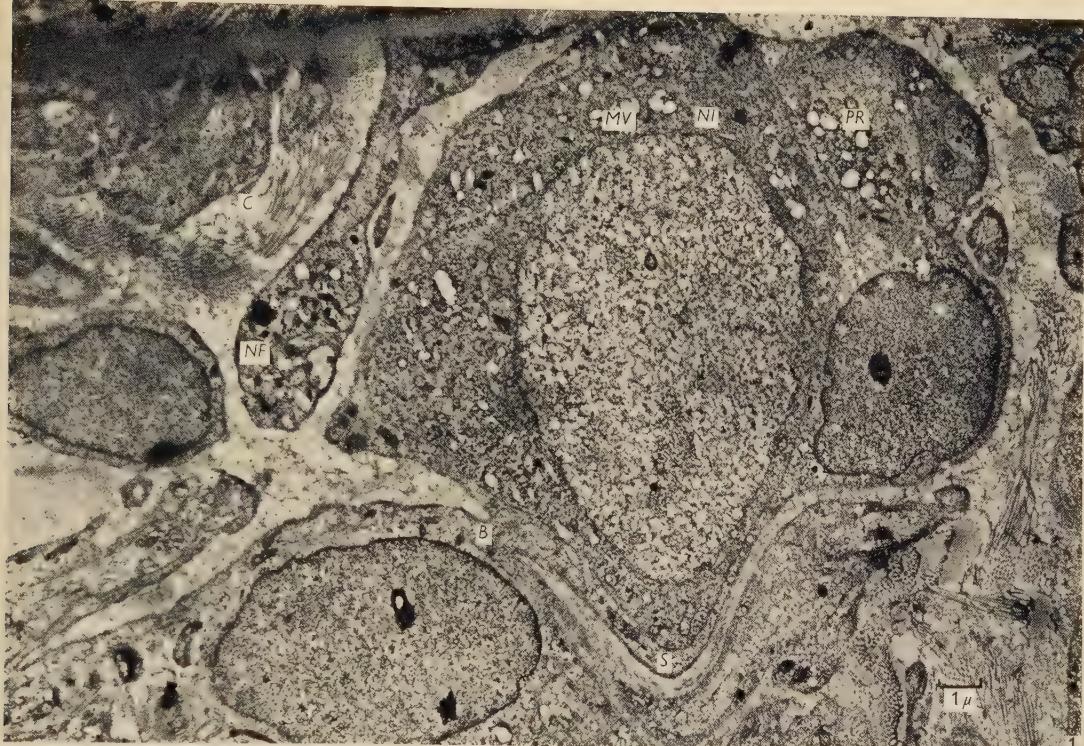
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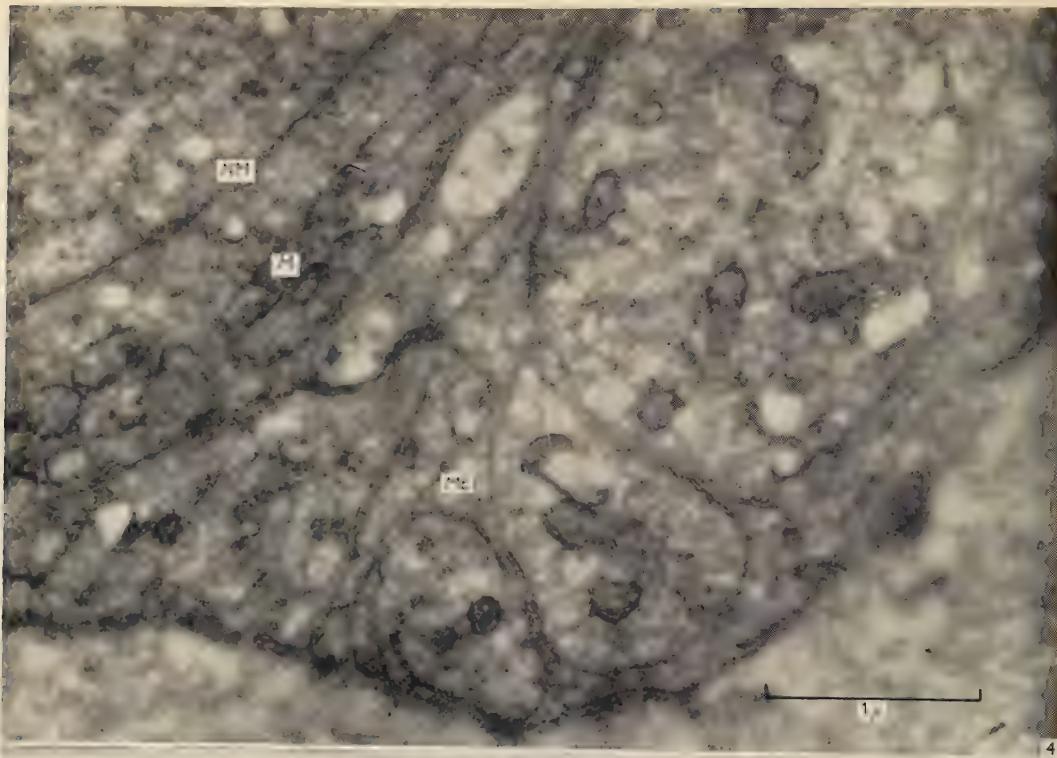
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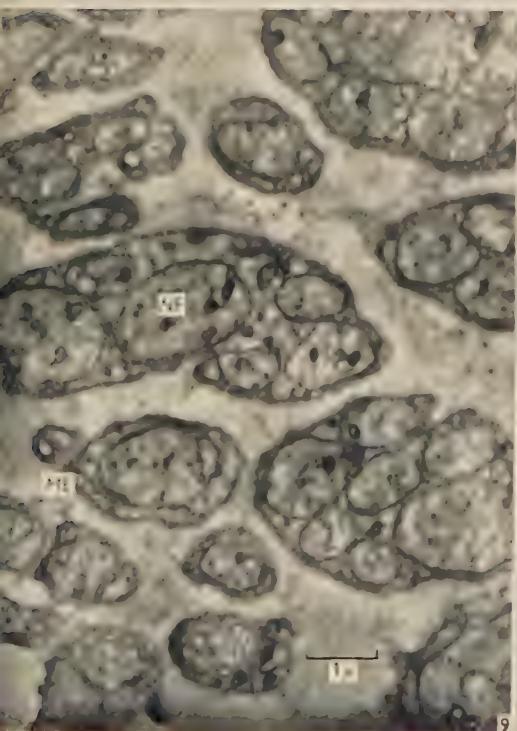
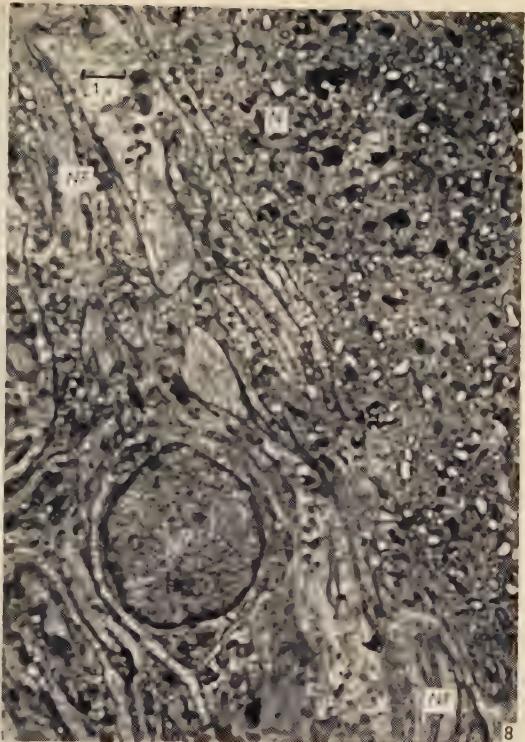
PLATE 1

Fig. 1. A normal neurone surrounded by collagen fibrils (C) and non-myelinated fibres (NF) lying within Schwann cells. The neurone contains vesicular mitochondria (MV) and Nissl substance (NI). It is surrounded by a satellite cell (S) which contains a neuronal process (PR) and is continuous with the Schwann cell at point B.

Fig. 2. Three neurones from a normal ganglion. They are surrounded by satellite cells containing nerve fibres (NF) in close association with the neuronal surface. Elongate vesicles associated with the Nissl substance (NI) and the clustered vesicles of the Golgi apparatus at G are also shown.









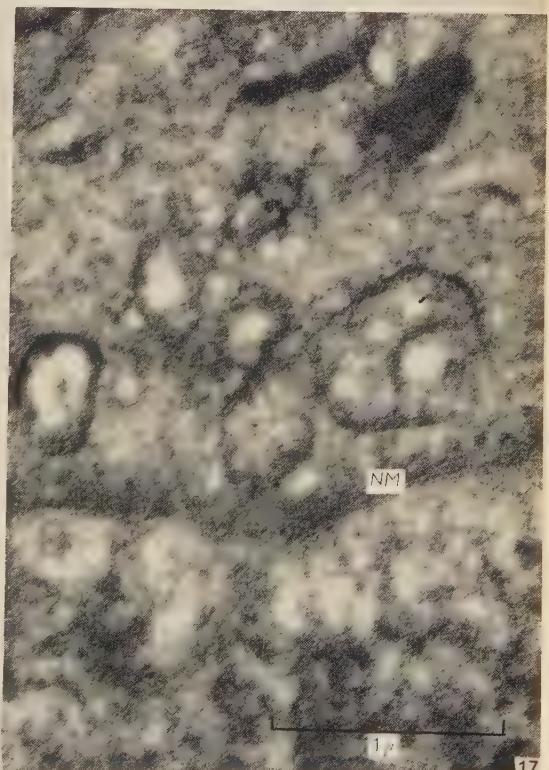
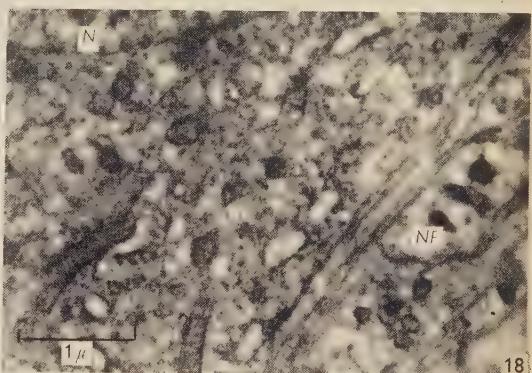
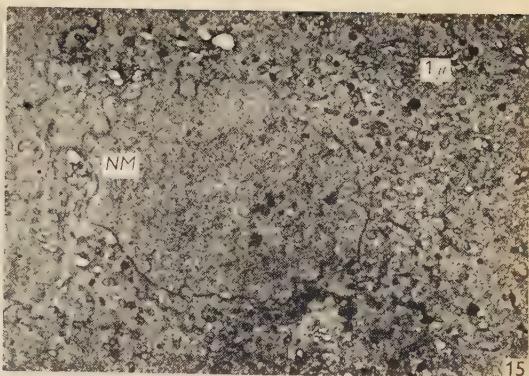


Fig. 3. Two blood vessels from the normal ganglion; one (*V*) consists of a single layer of endothelium. The other which contains a single red blood cell is composed of an inner endothelial lining and an outer layer of smooth muscle (*SM*) with bundles of collagen fibrils (*C*) in association with cells. A single nerve fibre is shown (*NF*).

PLATE 2

Fig. 4. A normal superior cervical ganglion showing the association of four nerve fibres with a satellite cell which lies on the surface of a neurone, its nuclear membrane (*NM*) and mitochondria (*M*) being shown. These nerve fibres lie at the bottom of invaginations of the inner layer of the satellite cell wall which form their mesaxons (*ME*).

Fig. 5. Part of the surface of a normal neurone. The appearance of the mitochondria and cytoplasm of the nerve fibre (*PR*) within the satellite cell surrounding the neurone *N* is similar to that of neuronal cytoplasm and it is thought that it forms one of its processes. In association with it are a number of small-diameter nerve fibres (*NF*).

Fig. 6. A normal neurone with a process (*PR*) in continuity with the neurone. The neuronal nucleus with nucleolus and nucleoplasm is surrounded by elongate mitochondria, Nissl substance (*NI*) and Golgi apparatus (*G*).

PLATE 3

Fig. 7. A group of nerve fibres 28 days after post-ganglionic section. There is a vesicular process at *PR* with a similar appearance to neuronal cytoplasm at this stage. Around it are otherwise normal nerve fibres (*NF*).

Fig. 8. Layers of cells and nerve fibres (*NF*) lying on the surface of a neurone (*N*).

Fig. 9. Three days after pre-ganglionic section the bundles of nerve fibres (*NF*) lying between neurones break up into smaller units. There is an increase in volume of the Schwann cells and it is easier to distinguish mesaxons (*ME*).

Fig. 10. Seven days after post-ganglionic section a Schwann cell has an irregular margin and contains masses of electron dense material.

PLATE 4

Fig. 11. Two neurones, 14 days after post-ganglionic section. The sprouts (*S*) formed at this stage are seen at the top right-hand and left-hand borders of the picture. There is a satellite cell nucleus at *SN* and small nerve fibres (*NF*) in the satellite cell cytoplasm.

Fig. 12. Fourteen days after post-ganglionic section the Schwann cells are in all degrees of association with the newly formed nerve fibres, and the cell at the lower border of the picture has a process which extends to enclose several nerve fibres (*NF*).

Fig. 13. A detail of the association of Schwann cell and nerve fibres 14 days after post-ganglionic section. The invagination of the cell wall to form a mesaxon is seen at *ME*.

PLATE 5

Fig. 14. A neurone and nerve fibres 7 days after pre-ganglionic section, the post-ganglionic fibres having been cut 7 days previously. The number of the fibres (*NF*) which resulted from the post-ganglionic section is undiminished. The majority of neuronal mitochondria are vesicular (*MV*).

Fig. 15. The nucleus of a neurone 48 hr. after post-ganglionic section. The nuclear membrane (*NM*) is convoluted. The cytoplasm appears to be normal, but, as frequently seen in material at this stage, the nucleus stands out less clearly.

Fig. 16. A neurone 28 days after post-ganglionic section. The nucleus is normal, but the cytoplasm contains many vesicular mitochondria (*MV*) and small aggregates of Nissl substance (*NI*).

Fig. 17. The nuclear membrane (*NM*) of a neurone 14 days after post-ganglionic section. Several vesicular mitochondria are shown and one appears to be forming from the nuclear membrane.

Fig. 18. A satellite cell 3 days after pre-ganglionic section, containing several relatively normal nerve fibres (*NF*). The neuronal nucleus (*N*) and cytoplasm are also shown.

THE HISTOCHEMICAL APPEARANCES OF CHOLIN-ESTERASE IN THE SUPERIOR CERVICAL SYMPATHETIC GANGLION AND THE CHANGES WHICH OCCUR AFTER PREGANGLIONIC NERVE SECTION

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The superior cervical sympathetic ganglion is an easily accessible part of the nervous system in which to study the histology and physiology of synaptic junctions. During the last twenty years a considerable number of new facts regarding the processes which occur at a synapse have been observed by electrophysiologists. Attempts to correlate histological findings with electrophysiological observations have, however, been hindered by the inability to stain synaptic terminals adequately. More than half a century has elapsed since Ehrlich (1886), Aronson (1886), Retzius (1889), Cajal (1893, 1903) and others carried out their pioneer histological studies on synaptic endings and the methods used during this time have changed only in the direction of refinement. During recent years much evidence has been produced for the existence of a membranous synaptic barrier in vertebrates and in some invertebrates. For the purposes of functional analysis it is desirable that more be known about the structure and histochemistry of this membranous barrier and its exact relationship to the axon terminals and the surfaces of the cells of termination. The present research was designed to investigate the histochemical appearances of cholinesterase in the superior cervical sympathetic ganglion and to study the changes which occur following section of the preganglionic trunk. It was hoped that the results obtained might provide new data to assist in the understanding of the mechanisms of synaptic function.

MATERIALS AND METHODS

Twelve mature cats were used for the investigation. The right sympathetic trunk of each animal was exposed under ether anaesthesia. The trunk was divided about 1 cm. below the superior cervical ganglion and the lower part in the neck was avulsed. The animals were killed in pairs 4, 6, 10, 20, 32 and 41 days after the operation. The right sympathetic ganglion and its appended pre- and postganglionic trunks were removed from each animal and at the same time the normal ganglion was removed from the left side of the neck to serve as a control. The ganglia were fixed in 10% formol saline for 15 hr. at room temperature, the formol saline having been previously neutralized over calcium carbonate. Longitudinal frozen sections 20μ thick were cut and washed in distilled water for 1 hr. The sections were then processed using a modification of the histochemical technique described by Koelle & Friedenwald (1949).

The optimum pH for the different types of cholinesterase has been reported to be in the region of 8.0 (Glick, 1938; Alles & Hawes, 1940). The original Koelle method fulfils adequately the requirements of enzyme specificity, but it is extremely sensitive and will demonstrate cholinesterase in those sites where it is in very low concentration. Furthermore, after the cholinesterase has split the substrate the products of hydrolysis diffuse to a small extent into adjacent regions until sufficient acidity has been built up locally to bring about precipitation of the copper thiocholine. In order to determine those parts of the ganglion in which the enzyme is most concentrated and at the same time to hasten the precipitation of copper thiocholine and so reduce diffusion artefacts, the hydrogen-ion concentration of the substrate was lowered to pH 4.2.

To distinguish between true and pseudo-cholinesterase the two substrates acetyl thiocholine iodide and butyryl thiocholine iodide were used. Acetyl thiocholine iodide is hydrolysed by both cholinesterases and butyryl thiocholine iodide is hydrolysed more rapidly by the pseudo-cholinesterase. The acetyl thiocholine iodide solution was made up as described previously (Snell & Garrett, 1957). The butyryl thiocholine iodide solution was made up in exactly the same manner, equimolecular concentrations of butyryl thiocholine iodide taking the place of acetyl thiocholine iodide.

In addition, a number of the ganglion sections were treated with substrate solutions also containing the following inhibitors: (1) eserine 3×10^{-5} M, to inhibit the activity of the cholinesterase group of enzymes while the simple esterases are practically insensitive; (2) BW 284 [1-5-bis(4 allyl-dimethyl-ammonium phenyl)pentan-3 one dibromide] 3×10^{-5} M, to inhibit true cholinesterase; (3) DFP [di-isopropylfluorophosphate] 3×10^{-10} M, to produce relatively selective inhibition of pseudo-cholinesterase. Sections were placed in an aqueous solution of the inhibitor at room temperature for 30 min. before incubation with the substrate plus the inhibitor at 37° C.

The ganglion sections were incubated with the substrate solutions alone or with the substrate solutions plus the inhibitor at 37° C. for 12, 18 and 24 hr. The concentrations of the inhibitors in the substrate solutions were the same as those given above. The sections were then washed in distilled water and placed in a 5% solution of ammonium sulphide for 2-3 min.; they were then washed again and mounted out of 70% alcohol in Canada balsam. Weak solutions of methylene blue or toluidin blue were used as counterstains.

The cholinesterase appearances in the superior cervical sympathetic ganglion were assessed by studying the concentration and distribution of the black copper sulphide precipitate formed. The effect of section of the preganglionic trunk on the cholinesterase appearances in the ganglion was studied by comparing sections of the ganglion on the operated side in each animal with those of the control ganglion of the other side, the sections having been incubated with the same substrate for exactly the same length of time.

RESULTS

Cholinesterase appearances in the normal superior cervical sympathetic ganglion

In the sections which had been incubated for 12 hr. cholinesterase activity was seen to be confined to fine nerve fibrils clustered around the ganglion cells. It was not possible to determine whether the enzyme activity was localized to the protoplasm of the axon of the fibril or to its surrounding sheath. No enzyme activity was seen in the ganglion cells or their processes. Furthermore, no cholinesterase activity was seen in the capsular cells or intercapsular glial cells and no activity could be detected in the nerve fibres entering or leaving the ganglion.

In the sections which had been incubated for 18 hr. the majority of the nerve fibres entering the lower pole of the ganglion showed a high cholinesterase activity which appeared to be situated mainly in the axons (Pl. 1, fig. 1). On tracing these fibres into the ganglion they were seen to give off numerous collaterals which became continuous with the fine fibrils clustered around the ganglion cells which had been observed in sections incubated for shorter periods. Lying along the course of the fibrils in the vicinity of the ganglion cells dark expansions were noted which had the characteristics of boutons de passage and boutons terminaux (Pl. 1, figs. 2, 3) described by previous workers. No cholinesterase activity was seen to be present in the ganglion cells or the proximal parts of their processes, in the capsular cells or in the intercapsular glial cells. Furthermore, no enzyme activity was seen in the nerve fibres leaving the upper pole of the ganglion.

In the sections which had been incubated for 24 hr., the precipitate of copper sulphide was much greater. The majority of the fibres entering the lower pole of the ganglion showed the same high cholinesterase activity (Pl. 1, fig. 4; Pl. 2, fig. 5). With this excessively long period of incubation the copper sulphide precipitate within the ganglion was gross and showed abnormal crystalline growth. It was possible, however, to visualize slight cholinesterase activity in the nuclei of the capsular cells. The nerve fibres leaving the upper pole of the ganglion showed no enzyme activity (Pl. 1, fig. 4; Pl. 2, fig. 6).

A comparative study of the sections which had been incubated with acetyl thiocholine iodide and butyryl thiocholine iodide and with substrate mixtures also containing the selective inhibitors DFP and BW 284 showed that both true and pseudo-cholinesterase activity is present in the nerve fibres entering the lower pole of the ganglion. It was found impossible by this method to assess the relative amounts of each type of enzyme present, especially in view of the very long incubation periods used. No esterase activity could be seen in the sections which had been incubated with substrate solutions also containing eserine.

Cholinesterase appearances in the superior cervical sympathetic ganglion following section of the preganglionic trunk

Although the animals were killed in pairs at 4, 6, 10, 20, 32 and 41 days after section of the sympathetic trunk the over-all picture of the cholinesterase activity in all the denervated ganglia was practically identical.

A macroscopic examination of the ganglion sections carried out by holding them

against a white background showed that the black copper sulphide precipitate formed as the result of enzyme activity was much less in the sections of the denervated ganglia which had been incubated with acetyl thiocholine iodide as compared with the controls. The reduced enzyme activity was most marked in those sections of the denervated ganglia which had been incubated in a substrate mixture containing acetyl thiocholine iodide and DFP. The sections of the denervated ganglia which had been incubated with butyryl thiocholine iodide also showed a slight reduction in enzyme activity as compared with the controls. This reduced activity was a little more marked in those sections which had been incubated with butyryl thiocholine iodide and BW 284. These observations indicate that preganglionic nerve section causes a marked reduction in the activity of true cholinesterase within the ganglion and a slight reduction in pseudo-cholinesterase activity.

In the sections which had been incubated for 12 and 18 hr. no cholinesterase activity could be seen in the degenerated nerve fibres entering the lower pole of the ganglion and no enzyme activity could be detected in the nerve fibres leaving the upper pole of the ganglion. Within the ganglion the enzyme activity was confined to the position formerly occupied by the fibrils clustered around and insinuated between the ganglion cells (Pl. 2, figs. 7, 8). The concentration of the copper sulphide precipitate appeared to be less than that seen in the controls. The cholinesterase activity was also clearly visible in the position formerly occupied by the boutons de passage and the boutons terminaux. As in the normal control ganglia no enzyme activity was seen in the ganglion cells or their processes or in the region of the capsular cells or intercapsular glial cells.

In the sections which were incubated for longer than 18 hr. the copper sulphide precipitate was greater but the enzyme activity remained confined to the degenerated fibrils clustered around the ganglion cells.

A comparative study of the sections which had been incubated with acetyl thiocholine iodide and butyryl thiocholine iodide and with substrate mixtures also containing the selective inhibitors DFP and BW 284 showed that both true and pseudo-cholinesterase activity was present in the degenerated fibrils around the ganglion cells. No esterase activity could be seen in the sections which had been incubated with substrate solutions also containing eserine.

DISCUSSION

The pioneer work of Ehrlich (1886), Smirnow (1890), Cajal (1893, 1903, 1905), Huber (1899) and Ranson & Billingsley (1918) revealed the fundamental structure of sympathetic ganglia. De Castro (1923) and Lawrentjew (1924) have since then contributed a vast amount of information concerning the detailed structure of sympathetic synapses. The greater part of these histological studies have been carried out by using silver impregnation methods.

Cajal (1893) described how in the nervous system most axons and their collaterals terminate at synapses by means of protoplasmic enlargements or boutons. Gibson (1940) gave an account of two forms of bouton in the superior cervical sympathetic ganglion of the cat—the bouton terminal and the bouton de passage. He found that the largest number of boutons seen in association with one cell or its processes

was thirteen. Each terminal bouton measured approximately $2\text{--}7\mu$ in diameter, and could be seen to be continuous with a very fine fibril. The boutons terminaux were found to lie in contact with the surfaces of the dendrites and the cell bodies. The boutons de passage rarely exceeded 2μ in diameter and were seen to be present in greater numbers. Gibson described how they were situated along the course of the finest preganglionic fibres at varying distances from their termination. He pointed out that they enable one fibre to make contact with many different ganglion cells or even with many points on the same cell. He found that the boutons de passage were subcapsular in position and made direct contact with the surface of the cell.

In recent years a number of electron microscopic studies have been made on the structure of synapses. De Robertis & Bennett (1954) studied the structure of synapses in the sympathetic ganglia of the frog. They found that the limiting membranes of the pre- and post-synaptic neuronal elements are 70–100 A.U. thick, separated from each other by an interneuronal space 100–150 A.U. wide. Causey & Hoffman (1956) have investigated the synaptic region of the superior cervical ganglion of the rabbit. They emphasize the difficulty in recognizing where exactly the preterminal axon actually becomes synaptic. However, their sections showed that at the sites where the axonal and ganglion cell membranes were in closest apposition they were separated by an interval of approximately 200 A.U. They also demonstrated that each presynaptic axon is enclosed by a membrane which is apparently derived from the surface membrane of a capsular cell. In fact, it would appear that each presynaptic axon has invaginated the surface membrane of a capsular cell and thus lies within the cell suspended from the cell boundary by a mesentery or mesaxon. The mesaxon is attached either to the outer free surface of the capsular cell or to the surface which lies in contact with the ganglion cell. This arrangement closely resembles the mesaxons derived from the Schwann cells seen in peripheral nerves (Gasser, 1952, 1955; Causey & Hoffman, 1956). It would thus appear that the environment of the finest terminal branches of the preganglionic fibres is that of the capsular cell cytoplasm. These findings are in agreement with the beliefs of de Castro (1951), who claimed that an adventitious element intervenes between the neuronal components of the synapse.

If the chemical mechanisms which take place at synaptic junctions in sympathetic ganglia are considered it is realized that a great mass of evidence has been adduced to show that acetylcholine is liberated in sufficient quantities to excite the ganglion cells when the preganglionic fibres are stimulated (Kibjakow, 1933; Feldberg & Vartiainen, 1934). The inference has been drawn that this substance is the synaptic transmitter. In spite of the strong arguments in favour of this theory it has been seriously challenged by many workers, and the evidence is reviewed by Eccles (1937). It is generally agreed, however, that acetylcholine probably plays a very important part in the process of synaptic transmission though it need not necessarily play the leading role. The acetylcholine content of sympathetic ganglia is relatively stable and it neither rises nor falls after long-lasting preganglionic stimulation (Minz, 1955). This constancy of level is presumably due to the presence of cholinesterase in high concentration within the ganglion which destroys excess acetylcholine by hydrolysis.

Koelle (1950) investigated the histochemical localization of cholinesterase in the

superior cervical sympathetic ganglion of the cat, employing incubating media buffered to pH 6.4. He found slight true cholinesterase activity in the cytoplasm and nuclei of most ganglion cells. The nuclei of numerous capsular cells and to a lesser extent their cytoplasm showed moderate true cholinesterase activity, as did many glial cell nuclei. True cholinesterase activity was also seen to be present in the entering preganglionic fibres. Superimposed on this background was the greater enzyme activity found in the Schwann's sheath nuclei and glial nuclei. Immediately adjacent to the ganglion cells there were noted fine wavy fibre-like deposits which ended in expansions suggestive of boutons terminaux. In the postganglionic trunk true cholinesterase activity occurred only in occasional sheath nuclei. He found that pseudo-cholinesterase activity was localized chiefly in the nuclei of the glial cells and ganglion cells and the cytoplasm of the latter showed varying degrees of activity. A small amount of pseudo-cholinesterase activity was also seen to be present in the Schwann's sheath nuclei of the pre- and postganglionic trunks.

Koelle found that in the ganglion of a cat which had undergone section of the cervical vago-sympathetic trunk 9 days previously, true cholinesterase activity was nearly completely absent. It remained very active, however, in occasional ganglion cells, while many others showed only faint traces of activity. The distribution and concentration of pseudo-cholinesterase activity were practically unaffected by this procedure.

In the present research a modification of the Koelle technique was used for the reasons explained previously and the sections were incubated with buffered substrate solutions at pH 4.2. The results showed that both true and pseudo-cholinesterase activity is present in great amount in the preganglionic nerve fibres situated in the preganglionic trunk and inside the ganglion. The absence of copper sulphide precipitate in the preganglionic fibres entering the lower pole of the ganglion and the presence of the precipitate in the fine terminal branches of the preganglionic fibres and boutons in the sections which were incubated for the minimum time of 12 hr. is an interesting finding. It would suggest that there is a local increase in the concentration of the enzyme in the terminal parts of the preganglionic fibres, although it may possibly be attributed to the absence of an insulating membrane in this region, thus allowing the substrate mixture greater access to the enzyme. Bartelmez & Hoerr (1933) showed that whenever presynaptic fibres are myelinated the myelin sheaths terminate abruptly a short distance proximal to the contact surface. It is unlikely that the latter explanation is responsible in this case since in histological sections many of the large preganglionic axons must be freely exposed to the substrate mixture and yet they showed a lower cholinesterase activity.

The denervation experiments showed that no cholinesterase activity was present in the degenerated preganglionic fibres entering the lower pole of the ganglion. A reduction in enzyme activity was to be expected since Snell (1957 *a, b*) showed histochemically, using the same method, that there is a marked fall in cholinesterase activity as nerve fibres undergo Wallerian degeneration. The observation that true cholinesterase activity within the ganglion is very much reduced following section of the preganglionic trunk and that pseudo-cholinesterase activity is only slightly reduced is almost certainly due to degeneration of the preganglionic fibres within the ganglion. Sawyer (1946) reported, as the result of manometric estimations, that in

nerves undergoing degeneration the true cholinesterase content becomes reduced by about 60% while the pseudo-cholinesterase remains practically unchanged.

Couteaux & Nachmansohn (1942) found by manometric estimation that the fall in the concentration of cholinesterase in the superior cervical ganglion of the cat following section of the preganglionic trunk occurs mainly in the first 24 hr., with some continued decrease after this. Ten to twelve days after the operation the total cholinesterase activity had become reduced to approximately 40% of its original value. Sawyer & Hollinshead (1945) carried out a similar investigation, but followed the disappearance of true and pseudo-cholinesterase individually. They found that the rapid decrease essentially concerns true cholinesterase and that pseudo-cholinesterase is lost to a much smaller extent and at a much slower rate. These results are in agreement with the histochemical findings of the present work.

The persistence of cholinesterase activity in reduced amount in the degenerated terminal branches of the preganglionic fibres, the boutons de passage and the boutons terminaux 41 days after preganglionic section is of special interest. De Castro (1930), Gibson (1940), and others, have clearly shown by means of silver stains that all the preganglionic fibres and boutons disappear in the superior cervical sympathetic ganglion of the cat by the 8th day following preganglionic section. A possible explanation is that the fine terminal branches of the preganglionic fibres and their boutons are surrounded by a high concentration of cholinesterase which is relatively unaffected by degeneration of the nerve fibres. If this is the case, it would seem likely that the enzyme is situated in the capsular cell cytoplasm or in the mesaxon derived from these cells which envelops the terminal branches of the preganglionic fibres, as described by Causey & Hoffman (1956). Another possibility is that this residual enzyme activity was confined to the axons derived from nerve cells lying within the ganglion and therefore unaffected by the preganglionic section. This was, however, discarded since the complexity and configuration of the fibre network resembled too closely that of the preganglionic fibres seen in the control ganglion. Unfortunately this method using frozen sections does not allow one to localize accurately the enzyme to the protoplasm of the axons or to the closely related capsular cells. Electron-microscopic observation of ultra-thin sections prepared from thick sections already processed by this method might give more precise information.

A similar persistence of cholinesterase is found at the motor end-plate of mammalian gastrocnemius muscles following section of the sciatic nerve. Couteaux & Nachmansohn (1940) reported that between the 3rd and 4th weeks following denervation the concentration of cholinesterase at the myoneural junction, as estimated manometrically, diminished by about 30–40% and thereafter remained at this level. Snell & McIntyre (1956) showed histochemically in the gastrocnemius muscle of the guinea-pig that the activity of cholinesterase gradually became reduced following denervation and at the 45th day after the operation was found to be absent. Recent work, using prolonged incubation periods, has shown, however, that a small amount of enzyme activity is still present at the motor end-plate 2 months after denervation (Snell, unpublished). Couteaux & Taxi (1952) have demonstrated by histochemical studies that the cholinesterase at the myoneural junction is situated chiefly in the muscular part of the end plate and is therefore almost exclusively concentrated outside the terminal branches of the motor nerve. There

would seem to be no reason why similar high concentrations of this enzyme should not exist around the terminal branches of preganglionic fibres at sympathetic synapses where so many other features of intercellular transmission run a parallel course to that found at the neuromuscular junction. The results of the present work certainly support this view.

The observation that the postganglionic axons exhibit no cholinesterase activity is in agreement with the histochemical results obtained by Koelle (1950). It is interesting to note in this connexion that Loewi & Hellauer (1938) showed that the preganglionic fibres of a sympathetic ganglion contain six times the acetylcholine content of the postganglionic fibres.

The absence of enzyme activity in the ganglion cells, capsular cells (a very small amount was seen in the nuclei after prolonged incubation) and Schwann sheath nuclei in the present work does not agree with the findings of Koelle (1950). This difference in the results suggests that the cholinesterase activity at these sites is very slight and cannot be demonstrated at the low hydrogen-ion concentration used in the present investigation.

SUMMARY

1. The histochemical appearances of cholinesterase in the superior cervical sympathetic ganglion were studied in twelve cats.
2. Cholinesterase activity was found to be high in the preganglionic fibres entering the lower pole of the ganglion, and the activity appeared to be greater in the terminal fibrils in the vicinity of the ganglion cells.
3. Both true and pseudo-cholinesterase activity were seen in the preganglionic nerve fibres. It was not possible to assess the relative amounts of each present.
4. No cholinesterase activity was seen in the ganglion cells or in the proximal parts of their processes. No enzyme activity was present in the capsular cells or intercapsular glial cells and no activity could be detected in the postganglionic nerve fibres leaving the upper pole of the ganglion.
5. Following section of the preganglionic nerve trunk the cholinesterase activity in the main preganglionic nerve fibres disappeared. Cholinesterase activity, however, persisted in reduced amount in the position formerly occupied by the terminal branches of the preganglionic fibres in the vicinity of the ganglion cells.
6. Macroscopic examination of the histological sections showed that pre-ganglionic nerve section causes a marked reduction in the activity of true cholinesterase within the ganglion and a slight reduction in the pseudo-cholinesterase activity.
7. The significance of these findings in relation to histological and physiological observations of past and present workers is discussed.

For this research I gratefully acknowledge the technical assistance of Miss J. M. Burke. My thanks are also due to Mr S. Hogwood for the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. High-power photomicrograph of longitudinal section of superior cervical sympathetic ganglion of cat. Shows high concentration of black copper sulphide precipitate in the pre-ganglionic nerve fibres in the region of the ganglion cells. The precipitate was formed as the result of cholinesterase activity. The round spaces are occupied by the ganglion cells. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 360$.

Fig. 2. High-power photomicrograph of nerve fibrils seen at the edge of a ganglion cell of superior cervical sympathetic ganglion of cat. Shows high concentration of black copper sulphide precipitate in the terminal branches of the pre-ganglionic nerve fibrils. The precipitate was formed as the result of cholinesterase activity. Note the bouton de passage (arrowed). Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 1055$.

Fig. 3. High-power photomicrograph of part of a ganglion cell of the superior cervical sympathetic ganglion of cat. Shows high concentration of black copper sulphide precipitate in the terminal branches of the pre-ganglionic nerve fibrils. The precipitate was formed as the result of cholinesterase activity. Note the boutons terminaux (arrowed). Acetyl thiocholine iodide was used as substrate. Counterstained with weak toluidin blue. $\times 1490$.

Fig. 4. Low-power photomicrograph of superior cervical sympathetic ganglion of cat. Shows high concentration of black copper sulphide precipitate in the pre-ganglionic fibres entering the lower pole of the ganglion. A very high concentration of copper sulphide is also seen within the ganglion. There is an absence of copper sulphide precipitate in the nerve fibres leaving the upper pole of the ganglion. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 14$.

PLATE 2

Fig. 5. Higher magnification of lower part of section shown in fig. 4. Shows a high concentration of black copper sulphide precipitate in the pre-ganglionic fibres entering the lower pole of the ganglion. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 123$.

Fig. 6. Higher magnification of upper part of section shown in fig. 4. Shows complete absence of black copper sulphide precipitate in the postganglionic fibres leaving the upper pole of the ganglion. This indicates that no cholinesterase activity was present in the postganglionic fibres. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 123$.

Fig. 7. Photomicrograph of longitudinal section of left (control) superior cervical sympathetic ganglion of cat. Shows high concentration of black copper sulphide precipitate in the pre-ganglionic nerve fibres in the region of the ganglion cells. The precipitate was formed as the result of cholinesterase activity. The round spaces are occupied by the ganglion cells. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 94$.

Fig. 8. Photomicrograph of longitudinal section of right (experimental) superior cervical sympathetic ganglion of cat. The sympathetic trunk was divided 1 cm. below the ganglion 3 weeks previously. The black copper sulphide precipitate is seen to be absent from the pre-ganglionic fibres lying at some distance from the ganglion cells, i.e. white tracts are apparent indicating an absence of cholinesterase activity. The precipitate is, however, present in the vicinity of the cells and was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. No counterstain. $\times 94$.



SNELL—HISTOCHEMICAL APPEARANCES OF CHOLINESTERASE

(Facing p. 418)



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THE EFFECTS OF HYPOTHYROIDISM ON THE DEVELOPMENT OF THE GROUND SUBSTANCE OF THE CEREBRAL CORTEX

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INTRODUCTION

Hypothyroidism produced early in the post-natal life of rats results in a closer packing of the neurones of the cerebral cortex. This change is attributed, not only to a decrease in size of the nerve-cell bodies but, in addition, to a decrease in the amount of substance lying between them (Eayrs & Taylor, 1951). In an attempt to account for these findings, attention was focused on the tissues lying between the perikarya. Barnett (1948) had, in fact, reported an impairment of myelination in the brains of young rats born of thiouracil-treated mothers, and it was subsequently shown (Eayrs & Horn, 1955; Horn, 1955) that there is a reduction in the density of the neuropil and a decrease in the length and amount of branching of the dendrites (Eayrs, 1955) in the cerebral cortex of hypothyroid rats. Since increase in size of the soma and development of axon-dendritic extensions are concomitants of differential growth, it would appear that thyroid deficiency leads to a retardation of neurone maturation.

If the central nervous system of an adult rat is treated according to the periodic-acid Schiff procedure, the stain is found between the nerve-cell bodies. Since this reaction is not exhibited by the perikarya, axons, dendrites or neuroglia, it was concluded that an intercellular matrix of a carbohydrate-protein nature occurs as a ground substance in the spaces of the neuropil, between the tissue and cellular elements of the brain (Hess, 1953).

Alterations in the ground substance of the cerebral cortex might contribute to the retarded growth and maturation of the neurones of hypothyroid rats in the following ways. In the first place, changes in the density of the ground substance might impair the nutrition of the nerve cell, since nutrient materials must pass from the capillary bed through the intercellular matrix in order to reach the neurone (Horn, 1955). Alternatively (Eayrs, 1955), or in addition, changes in the concentration of the ground substance might mechanically obstruct the development of nerve-cell processes.

The present investigation was therefore undertaken to determine the effects of thyroid deficiency, induced shortly after birth, upon the appearance and distribution of ground substance in the cerebral cortex of albino rats.

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MATERIAL AND METHODS*Experimental design*

A preliminary investigation was made to determine when the ground substance first appears and when it reaches the adult type of distribution in hypothyroid and normal rats. The actual experiment was designed on the basis of these results.

A total of seventy-seven animals were used, drawn from fifteen litters. Each experimental animal was paired with a normal litter-mate control of the same sex. A total of thirty-four litter-mate pairs were used, selected on a random basis from the fifteen litters and killed at varying intervals between the seventh and twenty-fourth post-natal days. Nine normal unpaired animals were killed at intervals between the time of birth and the age of 6 days.

Thyroid activity was suppressed on the day of birth by a single subcutaneous injection of $100\mu\text{c}$. of carrier-free radioactive iodine in the form of sodium iodide dissolved in normal saline (Goldberg & Chaikoff, 1949). The volume of solution injected was calculated from the decay curve of the isotope, and never exceeded 0.2 ml .

Histological methods

Animals were killed with chloroform. The brain and laryngeal region were removed, fixed in Rossman's fluid (absolute alcohol saturated with picric acid, 90 ml.; 40% solution of formaldehyde, 10 ml.) and embedded in paraffin. Sections of brain of 15μ thickness were prepared and stained by the periodic acid-Schiff technique as previously described (Hess, 1953). Brains from mothers of some of the litters were treated in a similar way to determine the distribution of PAS-positive material in the cerebral cortex of adult rats and so furnish a basis for comparing the appearance of the ground substance in the developing animals. Assessment of intensity of reaction to the PAS procedure was made independently by each observer, without knowledge of the age or experimental status of the animals from which the sections were taken. When each observer was satisfied with the consistency of his own results the data were compared. In those few instances where agreement was not achieved on first comparison, the slides were independently and randomly re-assessed until agreement on the rating to be assigned was reached.

The effectiveness of $100\mu\text{c}$. of ^{131}I in depressing the activity of the thyroid gland of newborn rats has previously been described (Horn, 1955). Nevertheless, the laryngeal region of each of ten randomly selected experimental animals, together with those of their normal controls, were serially sectioned at 10μ and stained with haematoxylin and eosin to show the presence or absence of thyroid tissue.

RESULTS(1) *Thyroid tissue*

No thyroid tissue was present in two of the ten experimental animals examined. The thyroid glands of the remaining eight animals were atrophic and grossly disorganized. In no instance were follicles found. The irregularly shaped parenchymal cells with dark pyknotic nuclei were arranged in whorls or cords in the connective

tissue stroma. In so far as histological evidence can be used as an index of function, it is concluded that in the animals receiving ^{131}I thyroid activity was severely depressed.

(2) *Ground substance of the cerebral cortex*

The distribution of the PAS-positive material in the cerebral cortex of the adult rat has previously been described (Hess, 1953). The grey matter reveals the unstained nerve cells embedded in a pink-staining homogeneous material. The white matter is uncoloured except for blood vessels.

The time of appearance and intensity of staining of the PAS-positive material was assessed in the cerebral cortex. In some of the lower centres of the brain, such as the caudate nucleus, differences were noted in the time of appearance and especially in the intensity of the reaction from those of the cerebral cortex, but were not studied in detail. Treatment with saliva had no effect on the subsequent PAS reaction in the brain of any of the animals.

The results are presented in Table 1. The amount of ground substance and the approximate degree of intensity of reaction to the PAS procedure were rated from 0 to 3+. The thickness of sections and the staining procedure were standardized as far as possible. Although the ratings were assigned by the subjective impression gained by visual examination, the results were quite consistent and indicate that the time of appearance and increase in quantity of the ground substance can readily be assessed.

(a) *Development of ground substance in the cerebral cortex of normal animals*

In newborn animals, no PAS reaction occurs between the cortical cell bodies. The cells are small and rounded and almost in contact with each other. This condition continues until about 3 days of age when the cells begin to elongate and separate from each other. At this time a very light PAS reaction occurs in the narrow spaces between the neurones; this reaction is rated 1+. No substantial changes are observed until about 8 days of age when the spaces between the cells are larger and the ground substance is increased in amount and stains more darkly. This reaction is rated 2+. After about 15 days of age, the PAS-positive substance consistently occurs in abundance between the neurones and stains relatively intensely. The reaction, although at times not as vivid as in adults, is still more intense than in most animals younger than sixteen days of age and is rated 3+. It thus appears that the ground substance of the cerebral cortex makes its first appearance in the brain of the rat at 3 days of age, increases in quantity during the second week of life, and at 16 days of age is similar in amount and in intensity of reaction to that of the adult. Illustrations of the ground substance when it is assigned its various ratings of amount and intensity are presented in Pl. 1, figs. 1-4.

(b) *Development of ground substance in the cerebral cortex of experimental animals*

No data are available on hypothyroid rats prior to the sixth day of age, when the rating is 1+. As in the controls, day 8 is the time of transition from 1+ to 2+, the latter rating persisting to the twelfth post-natal day in the experimental group,

when one animal is rated 2+ and another 3+. Thereafter there is a shift towards 3+. The period of transition from 2+ to 3+ extends from day 12 to day 17 in the hypothyroid group, compared with the 2-day period of transition, days 14 and 15, in the normal animals. There is thus more scatter among the experimental animals compared with their controls in passing from the 2+ to 3+ stage. Nevertheless, beyond day 17 the 3+ stage is reached in seven of the nine hypothyroid rats, the two exceptional cases having a 2+ rating.

Table 1. *Reaction to the PAS procedure of the ground substance of the cerebral cortex of hypothyroid and normal litter-mate control rats*

Age in days	No. of animals	Intensity of PAS reaction	
		Hypothyroid rats	Normal litter-mate controls
0	6	—	0
3	3	—	0
		—	+
6	6	+	+
		+	+
		+	+
8	6	+	++
		++	+
		++	++
9	6	++	++
		++	++
		++	++
10	6	++	++
		++	++
		++	++
12	4	++	++
		+++	++
13	2	+	++
14	8	++	++
		++	++
		+++	+++
15	6	++	++
		+++	++
		++	++
16	4	++	++
		++	+++
17	2	++	+++
18	2	+++	+++
19	2	+++	+++
20	4	+++	+++
		++	+++
21	2	+++	+++
22	6	++	+++
		+++	+++
23	2	+++	+++
		+++	+++

It is considered that there is no significant difference in the PAS rating between experimental and control groups and hence no difference in the time of appearance of the ground substance. Any apparent differences between the treatments may be accounted for on the basis of a greater variation to the PAS procedure among the hypothyroid rats compared with their controls.

DISCUSSION

From the present study it appears that thyroid deficiency does not cause any significant differences in the appearance or distribution of ground substance in the cerebral cortex of young rats. It is, of course, possible that changes in the ground substance will be found with the employment of more delicate quantitative methods than those used here. In addition, physical changes in polymerization and viscosity of the ground substance might occur and yet not be revealed by the histochemical techniques employed.

At both 15 and 24 days there is less interstitial tissue in the cerebral cortex of hypothyroid rats compared with their controls (Eayrs & Taylor, 1951); but there are no differences between the two groups in the intensity of reaction of the ground substance to the PAS procedure. The conclusions entailed by these apparently conflicting findings depend on whether the intensity of staining is determined by (a) the concentration of ground substance, that is the amount of PAS-positive material present per unit volume of interstitial tissue or (b) the total amount of ground substance present in the whole microscopic field examined. If the rating assigned is an index of concentration, failure to disclose differences in rating would indicate that the concentration of ground substance in the experimental group is not different from that in the controls. Alternatively, if the rating assigned is an index of the total quantity of ground substance present in the microscopic field, equal ratings imply equal amounts; but a given quantity of ground substance occupies a smaller volume in the hypothyroid rats than in normal animals; so the ground substance would be more concentrated in the former than in the latter. In practice, however, it seems likely that visual assessment is dependent on both concentration and amount of ground substance present. All that may be concluded on the basis of the present results is, therefore, that the concentration of ground substance in the cerebral cortex of hypothyroid animals is not less, but may be greater than that in normal controls. If there are no differences, however, it is necessary to abandon the hypothesis that the effect of thyroid deficiency on nerve-cell maturation operates through changes in the concentration of the ground substance.

In conclusion, it may be added that this study presents further evidence that the PAS reaction in the cerebral cortex is not due to the staining of nerve processes or their terminations, since in hypothyroidism the density of the neuropil is reduced, whereas the concentration of the ground substance is not.

SUMMARY

1. The development of the ground substance in the cerebral cortex has been studied in hypothyroid and normal litter-mate control albino rats.
2. Thyroid deficiency was induced shortly after birth by the subcutaneous administration of $100\mu\text{c}$. of radioactive iodine.
3. There were no significant differences in the time of appearance or distribution of ground substance between experimental and control animals.
4. These findings are discussed and it is suggested that the concentration of ground substance in the cerebral cortex of hypothyroid animals is not less, but may be greater, than that in normal controls.

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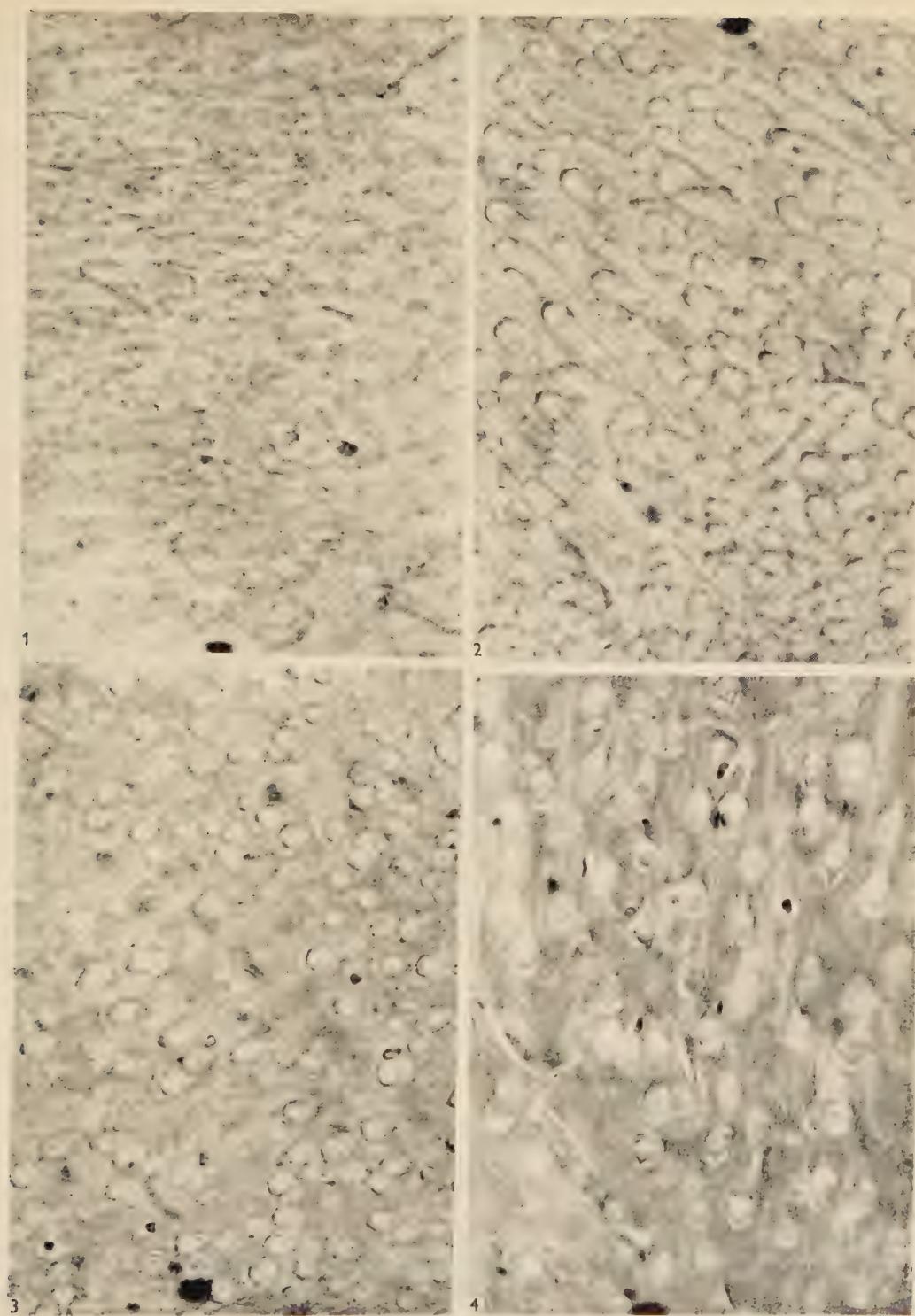
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EXPLANATION OF PLATE

All photographs are of Rossman-fixed paraffin sections of the cerebral cortex of normal rats stained by the periodic acid-Schiff procedure.

Fig. 1. New-born rat. Ground substance rating: 0.
Fig. 2. 3-day-old rat. Ground substance rating: 1+.
Fig. 3. 9-day-old rat. Ground substance rating: 2+.
Fig. 4. Adult rat. Ground substance rating: 3+.



HORN AND HESS—EFFECTS OF HYPOTHYROIDISM

(Facing p. 424)

DIFFERENTIATION OF CHROMOPHILIC AND CHROMOPHOBIC NEURONES

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INTRODUCTION

Neurones vary greatly in their capacity to be coloured by dyes. Chromophilic neurones, which stain intensely, are visible in the cerebrum and cerebellum (Cowdry, 1916) in addition to the more numerous chromophobic cells, which possess a lower affinity for tinctorial agents. Chromophilia is believed to vary inversely with the state of activity of individual neurones (see Einarson, 1949; Dixon, 1954; Einarson & Krogh, 1955); moreover, extreme loss of stainable cellular contents may occur as the result of neuronal injury and disease (Nissl, 1892; Marinesco, 1909; Spielmeyer, 1922; Einarson, 1949; Dixon, 1953b). The neurone is thus extremely labile in its power to be coloured by dyes, and changes in tingibility may give insight into the state of neuronal health and activity.

The study of chromophilia frequently involves the differential removal of basic dyes or of mordanted haematein from the cells. The difficulty inherent in methods dependent on differentiation was emphasized by Cowdry (1916). Even if progressive staining is employed, it is not possible to colour satisfactorily both chromophilic and chromophobic neurones in the same section (Miller, 1949). Einarson (1949), however, obviated this difficulty by using his elegant gallocyanin-chromalum technique; with this method he found that chromophilia varies with concentration of nucleic acid. Moreover, Dixon & Herbertson (1950) showed that chromophilic cells of the cerebral cortex and also some Purkinje cells in the cerebellum of rabbits stain intensely with the periodic acid-Schiff (PAS) method, while Dixon (1953a, 1954, 1957) found that chromophilic cells of both human and rabbit cerebral cortex contain a relatively high concentration of protein detectable by the tetrazo method of Danielli (1947).

The present communication shows that chromophilic neurones may be distinguished from chromophobes by their power to be coloured intensely by ferric chloride after previous treatment with acidified tannin. Although different reagents are employed, the technique used is the same in principle as the 'tanno-ferrique' method of Salazar (1920, 1921, 1944); this depends on preliminary attachment of tannin in acid solution to proteins in the tissues and the subsequent coloration of the bound tannin by iron.

MATERIALS AND METHODS

Rabbit cerebellum. The vermis of each cerebellum examined was sliced in the sagittal plane into two halves, which were immediately immersed in Carnoy's fluid. Tissue fixed in formol-neutral phosphate did not give satisfactory coloration with tannate iron.

Rabbit cerebrum. The cerebral hemispheres were cut in the coronal plane into thin slices and were then fixed in Carnoy's fluid.

Embedding, etc. Following fixation for 2 hr. the tissues were transferred to ethanol. After passage through three changes of ethanol (duration 6 hr. in all) the tissues were immersed in chloroform overnight; they were then transferred to wax (three changes over 6 hr.). Sections 5μ in thickness were cut, placed on slides, and incubated in an oven at 60°C overnight. This treatment firmly attached the sections to the slides. Sections used for the tannate iron and tetrazo methods were thus affixed to slides *without* employing any adhesive. For other methods albuminized slides were used.

Reagents for coloration by tannate iron

A. Tannic acid-HCl. Dissolve 10 g. tannic acid (British Drug Houses Ltd.) in distilled water; add 25 ml. N-HCl [concentrated HCl, sp.gr. 1.18, diluted to ten times its volume with distilled water], and make up to 250 ml. with distilled water.

B. Ferric chloride. Dissolve 10 g. analar $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water and make up to 250 ml. with distilled water.

Coloration of sections of brain by tannate iron

- (1) Remove wax with xylene. Wash briefly in ethanol, and then in running tap water for 5 min. Wash in distilled water.
- (2) Immerse sections in tannic acid-HCl in a Coplin jar for 10 min.
- (3) Wash for 1 min. in running tap water and rapidly rinse with distilled water.
- (4) Immerse sections in ferric chloride solution in a Coplin jar for 1 min. During this process the sections rapidly develop an inky black colour.
- (5) Wash rapidly in distilled water, followed successively by ethanol and xylene. Mount in DPX neutral mountant.

Tannic acid without added HCl

Instead of tannic acid-HCl a 4% (w/v) aqueous solution of tannic acid without added HCl was substituted in stage 2 of the method described above. Sections immersed in this solution developed only a comparatively feeble colour on subsequent immersion in ferric chloride.

Sections were also stained by Harris's haematoxylin and eosin. The periodic acid-Schiff (PAS) method of McManus (1948) without counterstain and the tetrazo method of Danielli (for technique see Dixon, 1953a, b) were used on some of the specimens.

RESULTS

Coloration of brain by tannate iron

After treatment with tannic acid-HCl the sections rapidly develop an inky black colour on immersion in ferric chloride.

Treatment with ferric chloride alone gives no colour to untanned sections; nor can tissue first immersed in ferric chloride be coloured more than very faintly by subsequent action of tannic acid-HCl, although a blue-black precipitate forms in the reagent.

Change during tanning. During treatment with tannic acid-HCl the sections assume a whitish opaque appearance probably due to the tanning of the tissue protein.

Effect of duration of tanning on subsequent coloration. Immersion for 5 min. in tannic acid-HCl enables almost full coloration to develop on subsequent transference to ferric chloride. The standard time for tanning is 10 min. Increase in the duration of tanning to 20 min., and even to 40 min., does not deepen the intensity of the subsequent coloration.

Duration of treatment with ferric chloride. The inky-black colour develops rapidly during the first few seconds in ferric chloride. The standard period of immersion in ferric chloride is 1 min.; immersion for 2 min. in ferric chloride does not result in any additional coloration.

Effect of added acid on tanning. The irreversible fixation of vegetable tannins by hide powder during short periods of tanning is highest at pH 1 (see Gustavson, 1949). For this reason tanning is carried out in a solution of tannic acid containing 0.1 N-HCl. Tannic acid solution not containing this added hydrochloric acid is comparatively ineffective. After tanning in such unacidified solutions for periods up to 40 min. the colour subsequently generated by treatment with ferric chloride is relatively feeble.

Effect of fixative. Sections of cerebellum fixed in formol-neutral-phosphate give poor coloration with tannate iron. Tissues fixed in Carnoy consistently develop a satisfactory colour.

Cerebellar neurones

Pl. 1, fig. 1, shows folia of the cerebellar vermis stained by tannate iron. The grey matter gives an intense reaction; the layer of granule cells is even more strongly coloured than the molecular layer.

Pl. 1, fig. 2, illustrates, at higher magnification, a portion of the same region shown in Pl. 1, fig. 1. It is evident from Pl. 1, fig. 2, that two types of Purkinje cells are present at the junction of the granular and molecular layers of the cortex; first, there are chromophilic cells which are stained an intense inky black, and secondly, there are grey chromophobic cells.

Pl. 1, fig. 3, shows, at still higher magnification, four chromophilic and one chromophobic Purkinje cells from the lowest part of the field shown in Pl. 1, fig. 2. The glomeruli of the granule cells also give a strong reaction with ferric tannate (Pl. 1, figs. 2, 3).

Pl. 1, fig. 4, shows cerebellar cortex, from the same specimen illustrated in Pl. 1, figs. 1-3, but stained by the tetrazo method; the same differentiation between chromophilic and chromophobic Purkinje cells is visible as with the ferric tannate technique. Sections of this tissue stained by PAS display a similar difference between chromophilic and chromophobic Purkinje cells. The glomeruli of the granule cells, which are so well coloured by tannate iron, also give a good reaction with the tetrazo (Pl. 1, fig. 4) and PAS techniques.

Careful inspection of the neuropil of the molecular layer, in sections stained by tannate iron, reveals the mitochondria as black granules and rodlets (Pl. 2, fig. 5); many of these are probably located in the dendritic branches.

Pl. 2, fig. 6, shows, under oil-immersion objective, part of the field shown in Pl. 2,

fig. 5. In Pl. 2, fig. 6, mitochondria may be discerned in the neuropil of the molecular layer and also in the cytoplasm of a chromophobic Purkinje cell. The intense reaction given by the chromophilic Purkinje cells precludes recognition of discrete mitochondria inside them.

The nuclear membranes, nucleoli, and chromatin of both the chromophobic Purkinje cells and the granule cells are well coloured by tannate iron. In the intensely stained chromophilic Purkinje cells nuclei are only visible with difficulty.

The white matter of the cerebellar folia is more feebly stained with tannate iron than the grey matter. The axons of myelinated fibres in white matter are, however, plainly visible, being coloured a greyish black. The ganglion cells in the roof nuclei of the vermis are also prominent, since their Nissl substance becomes grey in colour.

Cerebral cortex

Pl. 2, fig. 7, shows cerebral cortex stained by tannate iron; a clear differentiation is visible between the intensely coloured black chromophilic and the feebly tinted chromophobic pyramidal cells. The ground substance of the cerebral cortex is stained dark grey, probably mainly owing to coloration in the dendritic branches. The nuclear membranes, chromatin and nucleoli of the chromophobic pyramidal cells are strikingly revealed. In some of the chromophilic cells nuclei may be discerned; but the intense coloration of the cytoplasm in the latter cells generally obscures nuclear detail. The nuclei of glial cells are clearly visible.

DISCUSSION

Coloration of brain by tannate iron

Salazar (1920, 1921) described the 'tanno-ferrique' method of staining tissues. He treated sections with tannic acid followed by iron alum. Salazar clearly stressed that this method does not depend on the mere physical imbibition of preformed ferric tannate, but involves the coloration by iron of tannin already bound to the tissues. Salazar (1920, 1946) emphasized that the method only colours proteins; moreover, he found (1923) that the addition of acetic acid gives more rapid and powerful tanning. Tissues previously studied by his method include hypophysis, thyroid, stomach, pancreas and renal tubules (Salazar, 1944; Amado, 1954). The Golgi zones of cells (Salazar, 1944) and tannophilic chondriomes in the cytoplasm (Salazar, 1921) are also known to be stained by the tannoferric method, and in some cases nuclei react as well (Salazar, 1923).

In the sections of brain examined in the present study the coloured ferric compounds are visible at the loci of attachment of the tannin to the tissue proteins. This view is supported by the following evidence.

(i) Vegetable tannins are known to be fixed to a greater extent by hide powder at pH 1 than in less acid solutions (see Gustavson, 1949). With tannate iron a much more intense colour is finally developed in sections of cerebellum, when the preliminary tanning is carried out in the presence of 0.1 N-HCl, than in the absence of added mineral acid. Presumably the attachment of tannin to protein is greatest at low pH.

(ii) During the preliminary immersion in acidified tannic acid the sections of

cerebellum and cerebrum develop an opaque whitish colour, probably due to the precipitation of tannin on tissue proteins.

(iii) If ferric tannate is precipitated on sections of cerebellum by transferring them from ferric chloride to tannic acid-HCl, only a very faint coloration results. This implies that only tannin, which is first attached to constituents of the tissues, can later be coupled with iron so as to form a firmly held coloured complex. Salazar (1920) similarly showed that ferric tannate itself gives only a feeble or diffuse coloration to tissues.

According to Gustavson (1949), vegetable tannins are held by electrostatic attraction to oppositely charged NH_3^+ groups of the tissue proteins; co-ordination with peptide CONH groups may also contribute to the attachment of tannin to protein. The pronounced effect of added mineral acid, in enhancing the attachment of tannin in the tannate iron method, indicates that NH_3^+ groups are the major constituent of the tissues responsible for the binding of tannin. It is thus likely that this method reveals the location of NH_3^+ groups of proteins.

Positively charged metallic colloids are employed to locate *anionic* groups in polysaccharides and nucleic acids of tissues (see Hale, 1946; Wolman, 1956). With tannate iron, on the other hand, a *negatively charged anionic* colloid is used to reveal *kationic* groups in the tissue proteins; these *kationic* groups are located by their capacity to bind *negatively charged* colloidal ions of tannin, which are subsequently visualized by forming a black ferric compound.

Tannic acid is a vegetable tannin of high molecular weight; it contains carboxylic acid radicals, which may initially be bound by the NH_3^+ groups of the tissue proteins, and also phenolic hydroxyl groups, which can later react with ferric chloride to form intensely coloured complexes held to the tissue proteins. The sites of the kationic groups of the tissues are thus revealed with precision.

Chromophilic and chromophobic neurones

In the cerebellar cortex the chromophilic and chromophobic Purkinje cells are clearly differentiated by treatment with tannic acid followed by iron. The intense coloration of the chromophilic cells indicates that they contain more protein than the chromophobic cells (Pl. 1, figs. 2, 3; Pl. 2, figs. 5, 6). This view is confirmed by the results obtained with the tetrazo method (Pl. 1, fig. 4), which similarly reveals the greater concentration of protein in the chromophilic cells. Since the chromophilic Purkinje cells are only slightly smaller than the chromophobic cells, the extremely large difference between the concentration of protein in these two types of cell must reflect an absolute excess of protein in the former.

In cerebral cortex the chromophilic pyramidal cells are also much more intensely coloured than the chromophobic cells by tannate iron (Pl. 1, fig. 4). By use of the tetrazo method of Danielli, Dixon (1953a, 1954, 1957) established that the concentration of protein in chromophilic pyramidal cells is greater than in chromophobic. This distinction is now confirmed with tannate iron (Pl. 2, fig. 7). Chromophilic pyramidal cells, however, are considerably shrunken and are smaller than chromophobic cells. It is therefore uncertain whether the difference in staining between chromophilic and chromophobic pyramidal cells represents an absolute excess of protein in the former, as has been shown in the case of Purkinje cells.

Cowdry (1916) first drew attention to chromophilic Purkinje cells in the cerebellar cortex. Andrew (1956) confirmed the distinction between chromophilic and chromophobic Purkinje cells; he considered that these two types of cell lie in such close propinquity that no artefact of fixation can account for their difference in tingibility. Miller (1949) similarly excluded artefact as the cause of difference between chromophilic and chromophobic pyramidal cells. Tannate iron equally detects chromophilic and chromophobic neurones lying in proximity to one another in both cerebral and cerebellar cortex (Pl. 2, figs. 5-7). Similarly, adjacent chromophilic and chromophobic Purkinje cells are visible in sections of cerebellum stained by the tetrazo method and by Harris's haematoxylin and eosin.

Einarson & Krogh (1955) showed that increase in nervous activity causes loss of nucleic acid along with chromophobia. The present observations indicate that protein, detectable both by tannate iron and by the tetrazo method, is present in the chromophilic Purkinje cells in substantially greater quantity than in the chromophobic Purkinje cells. It is possible that this depletion of protein from the chromophobic cells is correlated with excessive nervous activity.

Black granules and rodlets are discernible in the chromophobic Purkinje cells and in the neuropil of the molecular layer of cerebellar cortex stained by tannate iron (Pl. 1, fig. 3; Pl. 2, fig. 6). Cowdry (1916) and Andrew (1956) showed that mitochondria, stainable by anilin acid fuchsin, occur in these situations. It is likely, therefore, that the mitochondria of neuronal cytoplasm are revealed by tannate iron. Salazar (1921) observed tannophil 'chondriomes' in the interstitial cells of the ovary, which he considered to be derived from mitochondrial substance.

Cowdry (1916) noted that mitochondria, stainable by anilin acid fuchsin, are fused into dense masses in chromophilic neurones; in some of these cells the darkly stained Nissl substance appears to be similarly conglomerated. With tannate iron the chromophilic Purkinje cells are so strongly coloured that cytoplasmic structure cannot well be discerned in them; their truly inky colour is associated with intense accumulation of intracellular protein.

Observations made with tannate iron confirm the view that the dendritic labyrinth of cerebral grey matter is rich in protein (Dixon, 1954). These results also indicate that protein is abundant in dendrites of cerebellar neurones. The enzymic nature of much of this protein probably accounts for the dominant position of the dendrites in cerebral metabolism.

SUMMARY

1. Chromophilic neurones are intensely coloured by ferric chloride after preliminary treatment with tannic acid in acid solution. This technique distinguishes chromophilic from chromophobic neurones both in cerebellar and cerebral cortex.
2. The method used is the same in principle as the 'tanno-ferrique' method of Salazar.
3. The method involves two successive reactions: first, negatively charged colloidal ions of tannin are attached to kationic groups of the tissue proteins; and secondly, the tannin so bound is visualized by subsequent treatment with ferric chloride, which generates an inky black colour in the tanned protein of the chromophilic neurones.

4. In the cerebellar cortex treatment with tannic acid followed by iron shows chromophilic and chromophobic Purkinje cells lying adjacent to one another. The chromophilic Purkinje cells contain substantially more protein than the chromophobes.

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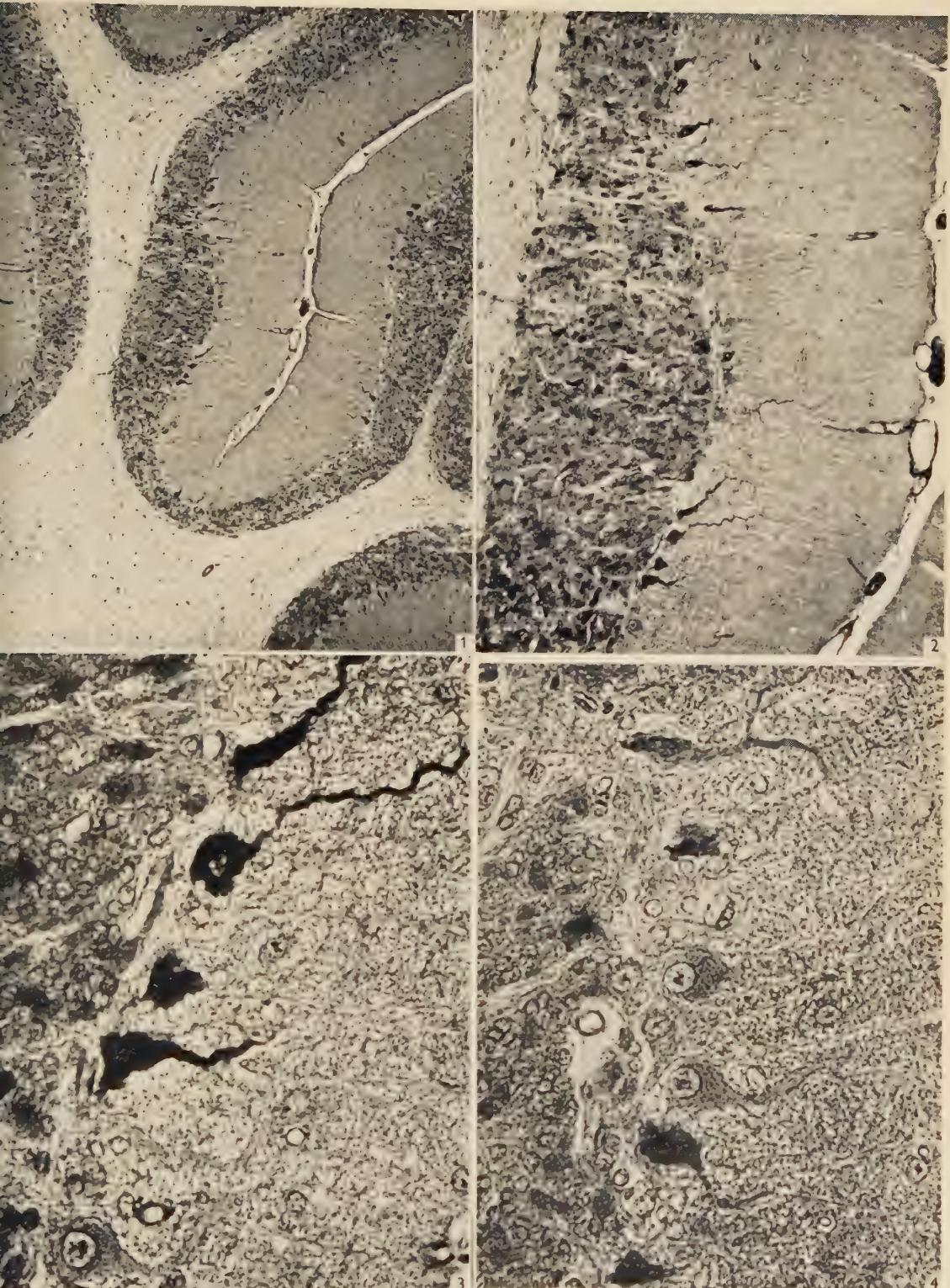
EXPLANATION OF PLATES

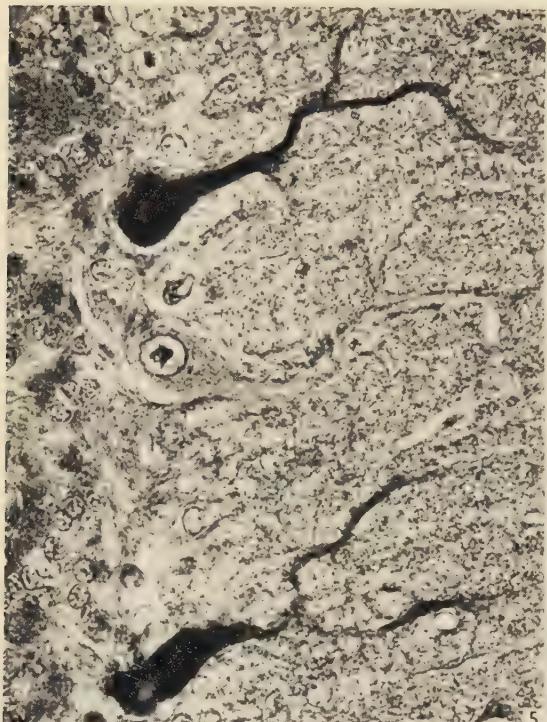
PLATE 1

Fig. 1. Cerebellar folia. Tannate iron. $\times 57$.
Fig. 2. Portion of field shown in fig. 2; black chromophilic and grey chromophobic Purkinje cells.
Tannate iron. $\times 104$.
Fig. 3. Portion of field shown in figs. 1 and 2; four black chromophilic and one grey chromophobic
Purkinje cells, also strongly stained glomeruli in granular layer. Tannate iron. $\times 410$.
Fig. 4. Same tissue as in figs. 1, 2, 3. Chromophilic and chromophobic Purkinje cells. Tetrazo.
 $\times 410$.

PLATE 2

Fig. 5. Two chromophilic and one chromophobic Purkinje cells. Tannate iron. $\times 410$.
Fig. 6. Two of the Purkinje cells shown in fig. 5; black mitochondrial granules and rodlets visible
in neuropil of the molecular layer. Tannate iron. $\times 1060$.
Fig. 7. Cerebral cortex; chromophilic and chromophobic pyramidal cells. Dark grey colour of the
cortical ground substance is probably due to reaction of the dendritic branches. Tannate iron.
 $\times 410$.





DIXON—DIFFERENTIATION OF CHROMOPHILIC AND CHROMOPHOBIC NEURONES

THE VASCULAR ARRANGEMENT OF THE MAMMALIAN RENAL GLOMERULUS AS REVEALED BY A STUDY OF ITS DEVELOPMENT

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INTRODUCTION

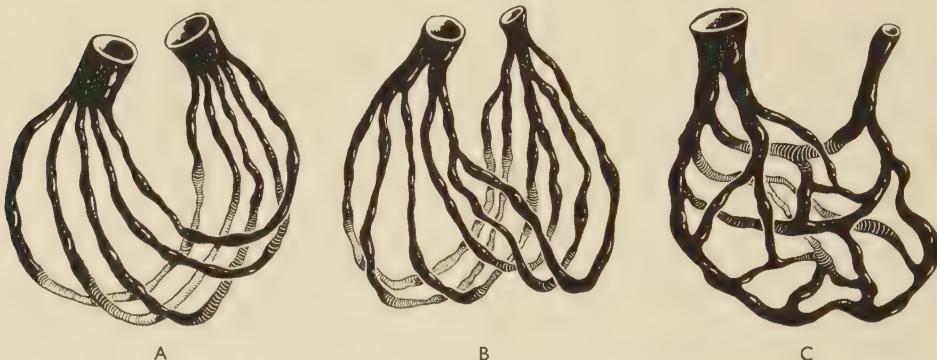
In recent years the fine structure of the renal filtration barrier has been revealed by electron microscopy (Yamada, 1955), but some conflict of views remains concerning a much grosser feature of glomerular organization, namely the arrangement of the capillaries constituting the glomerulus.

The view that the glomerulus consists of non-anastomosing capillary loops running from afferent to efferent arteriole has been enunciated by Bowman (1842). Ludwig (1872), Vimtrup (1928) and Wilmer (1941) using injection techniques, often combined with microdissection, have concurred with Bowman's findings, and this view is found in most text-books, Vimtrup being the author most frequently quoted. A few workers have described the glomerulus differently. Thus, Johnston (1899) has reconstructed a glomerulus, describing it as consisting of anastomosing capillaries, but this view has received little support. More recently Boyer (1956), using a plastic reconstruction method, has reached similar conclusions. Hall (1955), using latex injection combined with dissection and flattening of the glomeruli, has described the glomerulus as consisting of completely independent lobules each derived from a branch of the afferent arteriole and leading to its own branch of the efferent arteriole, though the capillaries within individual lobules freely anastomose. This view is rather a compromise between the other two (Text-fig. 1).

It is difficult to obtain a clear appreciation of the arrangement of such a small and complex structure as the glomerulus using any of these methods. Accurate reconstruction has many attendant difficulties and methods of injection combined with dissection and squashing lead to considerable distortion, and disruption of many vessels. The anatomy of such complex vascular beds can often be more clearly understood by studying their stages of development when the whole organ, or portion to be studied, is small and simple enough to be studied intact in a cleared preparation. Lewis (1958) has shown that the vessels of the renal glomerulus arise by the confluence of intercellular spaces appearing in a dense mass of mesodermal cells arising from the visceral layer of the developing Bowman's capsule. This study was undertaken to follow the development of the vascularization of these pre-glomerular masses of mesoderm and thereby to endeavour to obtain a clearer appreciation of the vascular arrangement of the glomerulus.

METHODS

The kidneys of foetal rabbits, sheep, and guinea-pigs, injected with Monastral Fast Blue B.N.V.S. Paste, were used, and also those of adult rabbits injected with Indian ink or Monastral Fast Blue. The kidneys were fixed in Bouin's fluid, dehydrated, embedded in celloidin and sectioned at 40μ or 100μ thickness. The sections were cleared in methyl benzoate, mounted and examined with a Zeiss Stereo-microscope giving a magnification of $\times 160$, enabling clear three-dimensional visualization of the developing glomeruli. Specific points in the vascular arrangements were checked using a binocular microscope with a long working-distance $\frac{1}{6}$ in. objective.



Text-fig. 1. A simplified diagrammatic representation of the varying views on glomerular capillary organization. (A) The glomerulus considered as consisting of non-anastomosing loops arising directly from the afferent arteriole. This view is often presented in text-books as representing Vimtrup's view. (B) A more accurate representation of Vimtrup's view with the afferent arteriole arborizing before giving rise to non-anastomosing loops which reform the efferent arteriole in similar fashion. (C) The concept of the glomerulus consisting of an anastomosing plexus of capillaries (according to Johnston and to Boyer).

OBSERVATIONS

The following description of the sequence of developmental changes in the glomerulus is applicable to any of the three species examined (rabbit, guinea-pig, sheep) for in all of these the process was similar. In any one section of a developing metanephros all stages of canalization and resultant vascularization of the dense mesodermal precursors of the glomeruli can be seen, the youngest and least complex being close to the surface neogenic zone, the more complicated and highly vascularized glomeruli being deeper in the cortex. Intercellular spaces develop in the pre-glomeruli and unite to form the lumina of the glomerular vessels which then become confluent with the extra-glomerular cortical sinusoids, which develop similarly in the mesenchyme surrounding the tubules and Bowman's capsules. These latter connexions will become the afferent and efferent arterioles (Lewis, 1958). The earliest stage of vascularization of the originally solid, cellular, pre-glomerulus shows the formation of a wide-bored channel at the periphery of the mass, into one end of which opens the developing afferent arteriole, while the other end is continued as the more slender efferent arteriole joining the surrounding cortical vascular plexus (Pl. 1, fig. 1).

The large peripheral channel is crescent-shaped with its afferent and efferent ends close together, marking the future vascular pole of the glomerulus. At this position, before vascularization, the solid mesodermal mass of the pre-glomerulus is continuous with the surrounding mesenchyme with its developing cortical vessels, while the remainder of the glomerulus is covered by the visceral layer of Bowman's capsule.

Canalization of the central parts of the pre-glomerular mass proceeds in a similar manner with the formation of connecting channels penetrating the initially solid core of the glomerulus from the point of entry of the afferent arteriole to the peripheral channel. Other peripheral channels may often be forming by a similar process at this stage close to the surface of the spherical glomerulus, anastomosing with the original peripheral channel to form the beginnings of a peripheral plexus at the surface of the glomerulus (Pl. 1, fig. 2). The peripheral channel (or channels) soon becomes indented at the points where it is joined by the narrower channels traversing the core, giving it a sinuous form. The scalloped surface so formed produces a commencing lobulation of the glomerulus (Pl. 1, fig. 3). The newly formed core vessel leading from the afferent arteriole and joining the peripheral channel nearest its efferent end is the largest (of similar diameter as the peripheral vessel) and so a wide-bored loop of sinuous form situated at the end of the afferent arteriole is formed. This loop leads from the afferent arteriole to the peripheral channel and back through the large core channel, to the *afferent* arteriole. The remaining narrower vessels, formed in the core, traverse its interior (Pl. 1, fig. 3). Incomplete injection may not fill the core vessels, only the wide-bored loop being filled (Pl. 1, fig. 5). The picture of such complete loop formations at the ends of the terminal branchings of the arterial tree is a conspicuous feature of the injected foetal kidney. The efferent arteriole is a comparatively small and inconspicuous channel arising from the loop and passing to join the cortical capillary plexus, or giving rise to *vasa recta* in the case of a juxta-medullary glomerulus (Pl. 1, figs. 4, 6).

The beginnings of the next phase have already been noted. As vascularization proceeds other peripheral vessels are elaborated and a rind of anastomosing vessels constituting a peripheral plexus is produced at the surface of the glomerulus (Pl. 1, figs. 7, 9; Pl. 2, fig. 13). At the same time the system of connecting vessels in the core of the glomerulus is being elaborated further and the whole glomerulus thus has the arrangement of an anastomosing plexus (Pl. 2, fig. 15). The afferent arteriole, upon entering the glomerulus, presents a dilatation from which both peripheral and central branches arise (Pl. 1, figs. 5, 8).

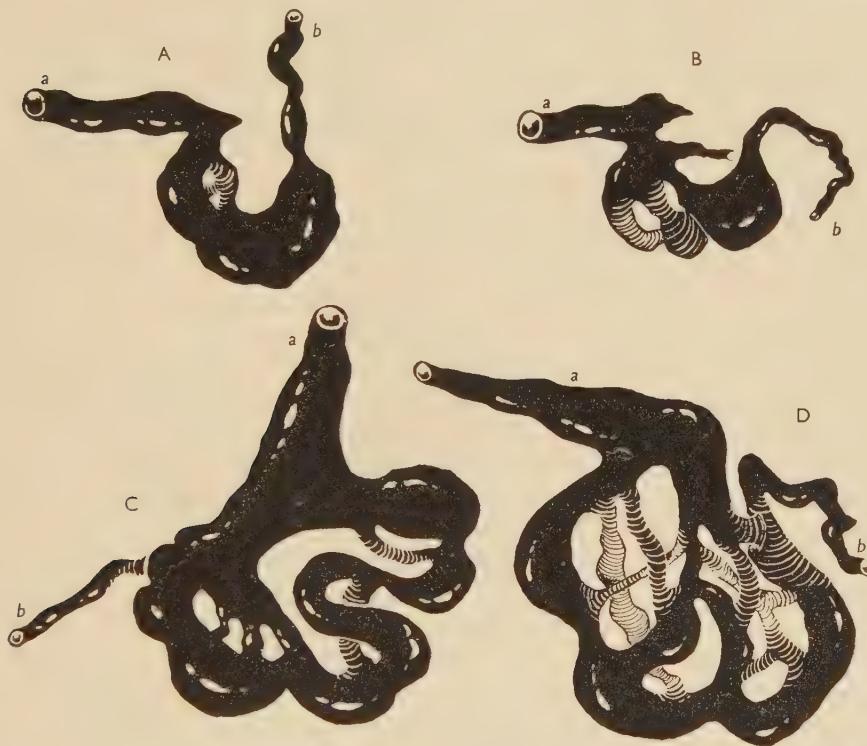
These processes continue, progressively elaborating the anastomosing rind of peripheral vessels and also the system of small channels joining this plexus through the core of the glomerulus. The tortuous form of the peripheral vessels persists, the indentations giving rise to the lobulated form. The efferent arteriole is small and inconspicuous compared to the afferent arteriole. The fundamental pattern is unchanged from the earlier stages but even now the vascular arrangement is becoming so complex that it would be difficult to understand clearly without a knowledge of the earlier stages (Pl. 2, figs. 11, 14, 16). The indentations in the peripheral channels are often quite deep, giving rise to an appearance of deep fissuring with lobulation, but the lobules are not separate and distinct, for the peripheral vessels do run across from one lobule to the other in the depths of the fissures (Pl. 2, fig. 12).

A study of adult rabbit kidneys, injected with Indian ink or *Monastral Fast Blue*, left no doubt that the fundamental glomerular vascular pattern as described for the foetus persists in the highly complex adult glomerulus. The greater diameter of the lumen of the peripheral vessels relative to that of the core vessels is most marked in the earliest stages of development, though even in the adult the peripheral channels are still somewhat larger.

CONCLUSIONS

Vascularization of the glomerulus consists essentially of the formation of inter-cellular spaces in a solid mass of cells producing the lumina of vessels. New knowledge of the fine structure of the glomerulus as shown by electron microscopy (Yamada, 1955) can thus be readily interpreted. Yamada has shown the adult glomerulus to consist essentially of a cellular mass containing lumina whose lining cells are endothelium and the intervening cells are the intercapillary cells or mesangium. The whole glomerulus, including the free surfaces of the capillaries not related to mesangium, is clothed by the specialized epithelial cells or 'podocytes'. It can be seen how, during development, those cells adjacent to the lumina formed in the solid precursor of the glomerulus must form endothelium, the remainder forming mesangium, while the originally cuboidal epithelium covering the glomerulus, the visceral layer of the embryonic Bowman's capsule, flattens forming the podocytes with their inter-digitating processes. Vascularization forms initially a wide crescentic channel close to the glomerular surface connected at its ends to the afferent and efferent arterioles. Channels are very soon formed traversing the initially solid glomerular core from afferent arteriole to peripheral channel and the appearance of a wide-bored loop at the end of the arterial branch, with the centre of the loop traversed by finer core channels, is soon produced. The efferent arteriole leaves the loop part way around. Further peripheral channels are soon formed, producing a plexus at the surface joined on its inner side by the connecting core channels. Even the first formed peripheral channel soon gains a sinuous, indented form producing an early appearance of lobulation. The complexity increases greatly up to the adult, but the fundamental form remains unchanged. The surface indentations may become quite deep, but the lobules produced are not independent and distinct, the peripheral channels being continuous from lobule to lobule in the depths of the fissures. These changes are illustrated in Text-fig. 2. Although the peripheral channel becomes a plexus, by considering any one plane, the glomerulus can be looked upon as a loop beginning and ending in the dilatation of the afferent arteriole and with somewhat finer channels traversing its interior. The arrangement as described is reasonable on comparative grounds. Bowman (1842) described the bird and reptile metanephric glomerulus as a single, undivided, dilated and coiled vessel, but Vilter (1935) has clearly shown that in the pigeon it consists of a plexus of vessels ramifying over the surface of a solid, central cell mass. Whether this type represents a regressive change associated with a reduction in water excretion or a truly more primitive form, its close relationship to the mammalian pattern as described, is obvious. The basic glomerular pattern then consists of the peripheral channels forming a plexus which can be considered as made up of complete but anastomosing loops

beginning and ending in the dilatation of the afferent arteriole (Pl. 1, fig. 5), while the efferent arteriole is a rather small effluent channel leading out of this system. In the mammal there is the addition of vessels traversing the core.



Text-fig. 2. Four stages in the vascularization of a glomerulus. The figures, although semi-diagrammatic, were drawn from actual glomeruli with the aid of a camera lucida. (A) A very early stage of vascularization, showing the afferent arteriole (*a*), the efferent arteriole (*b*), the large crescentic peripheral channel, and two small core vessels already formed. (B) A slightly later stage. The peripheral plexus is becoming multiple. A core vessel is seen not quite reaching the peripheral plexus, or not fully injected. (C) A later stage. The looped arrangement formed by the peripheral vessel is shown and it has a deep indentation in it producing lobulation of the glomerulus. (D) A later stage. The fundamental adult pattern is already established although complexity is not yet very great.

The picture of vascular organization here described agrees well with Johnston's very careful reconstruction of a single glomerulus. This work has received very little recognition and has been strongly denied by Vimtrup (1928) who has championed the original view of non-anastomosing loops. Vimtrup has reached his conclusions by dissecting the glomeruli of adult kidneys, a difficult procedure which could readily lead to incorrect conclusions. Wilmer (1941), using a celloidin injection-corrosion technique, has noted the branching of the afferent arteriole with an arborization of primary, secondary, etc., branches, and has also described the surface view with the convexities of the loops of the serpiginous vessels. However, he draws the unwarranted conclusion that the afferent branches are joined to the loops which

then pass to the efferent arteriole forming a system of non-anastomosing capillaries. Vimtrup figures the afferent arteriole breaking immediately into the full complement of non-anastomosing capillary loops and this figure is frequently reproduced (Ham, 1953). However, Vimtrup states that this figure is diagrammatic, being intended to show his concept of the independent arrangement of the capillary loops, and amplifies his description by another figure showing the actual arborizing manner in which the afferent arteriole breaks up, the efferent arteriole being reconstituted similarly. Vimtrup's figure showing the immediate breaking up of the afferent arteriole into the full number of capillary loops would obviously be totally irreconcilable with the description given here, but when it is realized that Vimtrup actually did observe an arborizing branching of the afferent arteriole, the divergence of views is not so great (Text-fig. 1). Incomplete injections of glomeruli, with their vascular arrangements as described in this paper, do show an arborizing division of the afferent arteriole, and it appears that Vimtrup and Wilmer, while observing this correctly, drew the erroneous conclusion that the branches then passed into a series of independent capillary loops. Hall (quoted by Maximow and Bloom, 1957) has recently produced a new view. Within any one lobule he describes a pattern fundamentally as that described in this paper for the whole glomerulus, but maintains that the lobules are separate and distinct anatomical units. The larger communicating vessels of Hall's description undoubtedly correspond with the larger peripheral channels here described, but the dissecting and squashing technique which he has applied to the injected glomeruli has undoubtedly disrupted the continuity of these channels. Johnston (1899) and Boyer (1956) have also found that the lobules are not independent, having anastomotic vessels bridging the fissures between them. Thus, embryological study supports Johnston's long neglected work and also throws new light on the vascular organization of the glomerulus.

While the glomerulus has been considered to consist of non-anastomosing loops an obstructive action of the small efferent arteriole has been thought to be the important factor in maintaining pressure along the length of these loops, and some doubt has been expressed as to whether or not there was some reabsorption of filtrate near the downstream end of the vessels (Fulton, 1949). The arrangement described here provides a more perfect filtering mechanism—a loop (Pl. 1, fig. 5) at the end of an afferent arteriole would provide an almost uniform pressure around it, with little fall downstream, the whole length of the loop then being utilized as an effective filter. The glomerulus, as has been seen, is just a great elaboration of such a looped system (the peripheral plexus) with the addition of core channels pouring fresh blood into the loops at intervals. The efferent arteriole is probably best looked upon as a vessel constantly drawing off concentrated blood from the system, its small size being a result rather than a cause of the filtration.

The large channels, or one of them, of the peripheral plexus may exist as shunts or more direct channels through the plexiform glomerulus from afferent to efferent arteriole. This would appear to be more important in the foetus where the peripheral channels are larger. It is of interest that direct observation of living frog kidneys (Bieter, 1930), with stimulation of the splanchnic nerves, has shown what appear to be similar shunt channels operating in some glomeruli.

SUMMARY

1. The development of the mammalian renal glomerulus is described in several species.
2. The precursor of the glomerulus is a solid sphere of mesodermal cells in which vessels develop by canalization.
3. The glomerulus comes to consist of an anastomosing plexus of vessels possessing a complex but definite arrangement, and not of independent capillary loops.
4. The lobules of the glomerulus are not independent.

I would like to thank Prof. D. V. Davies for helpful criticism, Mr G. Maxwell for technical assistance and Mr J. S. Fenton for the photographs.

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EXPLANATION OF PLATES

All the sections are from kidneys injected with Monastral Fast Blue B.N.V.S. Paste, $\times 320$.
a, afferent arteriole; *b*, efferent arteriole.

PLATE I

Fig. 1. A very early and simple 27-day foetal rabbit glomerulus. It consists of a wide semilunar channel into one end of which leads a large afferent arteriole and out of the other end a slender efferent arteriole. Some of the surrounding cortical sinusoids are shown.

Fig. 2. 27-day foetal rabbit glomerulus. The peripheral channel is in part double (not very clear in the photograph) and the slender core vessels leading from afferent arteriole to the peripheral vessels are seen.

Fig. 3. 27-day foetal rabbit glomerulus. The serpiginous outline of the peripheral channel (partially double) and the slender core vessels joining it are shown. The origin of the efferent arteriole cannot be seen in the photograph.

Fig. 4. 27-day foetal rabbit glomerulus showing the serpiginous peripheral channel (in part double), slender core vessels, and the origin of the efferent arteriole.

Fig. 5. An incompletely injected 27-day foetal rabbit glomerulus. The afferent arteriole shows a dilatation at its point of entry into the glomerulus and the peripheral channel, double in part, and with an indented appearance, forms a loop beginning and ending in the afferent arteriole. The efferent arteriole is not shown.

Fig. 6. 27-day foetal rabbit glomerulus (incomplete injection). The peripheral vessels are multiple above but in general form a loop arising and ending in the dilatation formed by the afferent arteriole. The efferent arteriole, in this case an arteriola recta entering the medulla, arises from this peripheral channel.

Fig. 7. 27-day foetal rabbit glomerulus showing the plexiform arrangement of peripheral channels at the glomerular surface.

Fig. 8. An incompletely injected 27-day foetal rabbit glomerulus. The view is taken looking through the glomerulus towards the vascular pole with the afferent arteriole entering from below the field of view and dilating before giving off its primary branches which further subdivide.

Fig. 9. Two 27-day foetal rabbit glomeruli showing the anastomosing arrangement of the peripheral vessels at the surface.

Fig. 10. Two 27-day foetal rabbit glomeruli. The upper shows the afferent arteriole entering from below, dilating and giving off slender core vessels radiating out to join the serpiginous peripheral channel. There is a deep indentation in the peripheral channel of the lower glomerulus.

PLATE 2

Fig. 11. A rather highly elaborated 27-day foetal rabbit glomerulus.

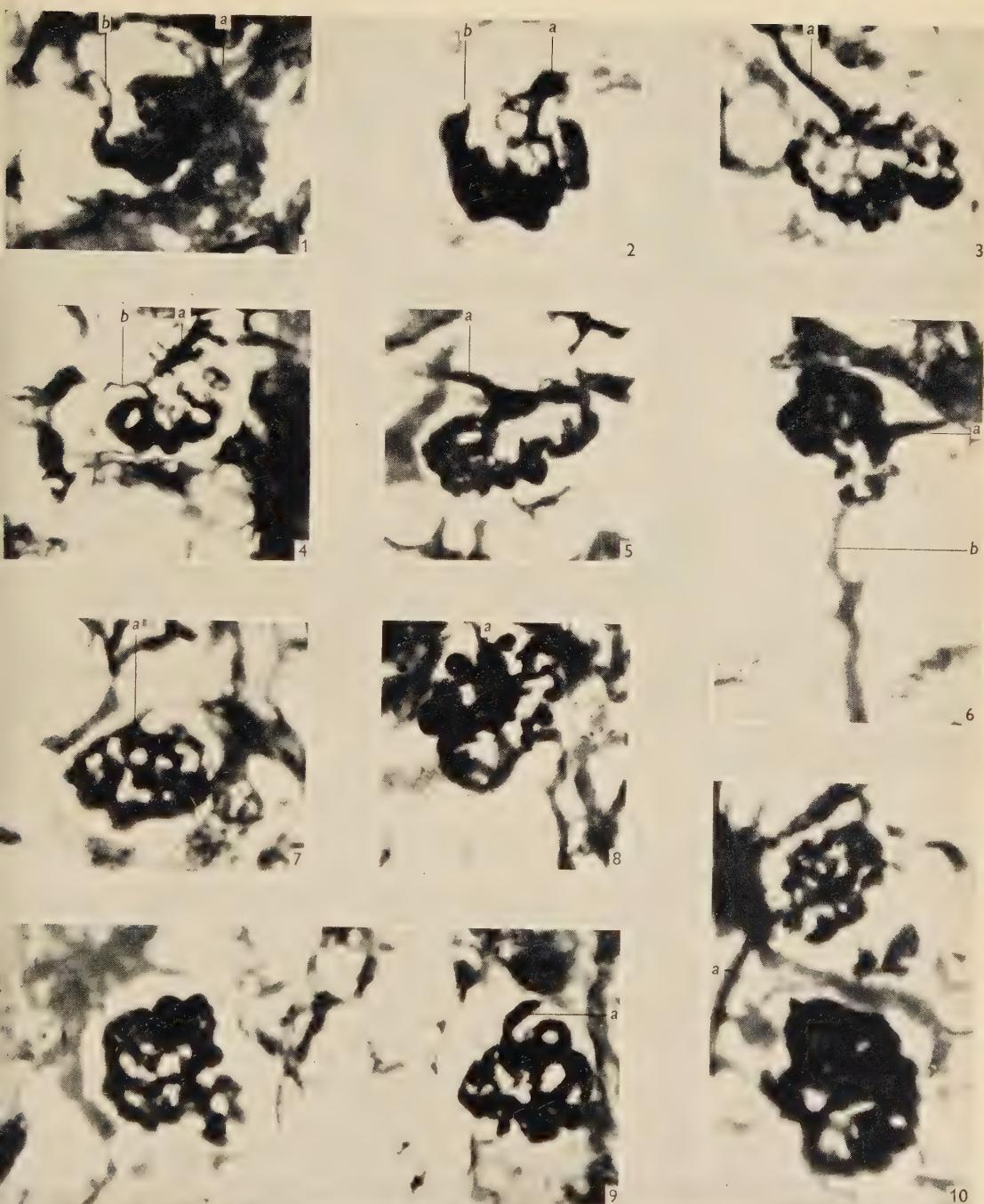
Fig. 12. A 27-day foetal rabbit glomerulus. The plexiform anastomosing character of its vessels is clearly shown. There is a deep fissure subdividing it into two main lobules (large and small) but these are not independent for a large peripheral vessel passes from one to the other in the depths of the fissure, which is only an indentation in it.

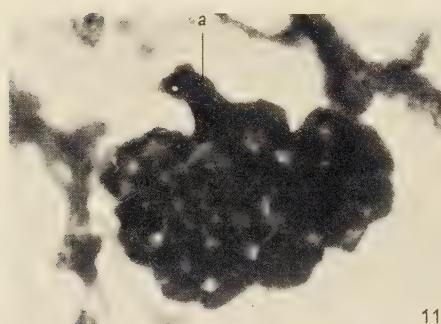
Fig. 13. Two 90 mm. foetal guinea-pig glomeruli showing the plexiform rind of peripheral vessels.

Fig. 14. A fairly elaborate 22 cm. foetal sheep glomerulus. The peripheral plexus and some of the core channels are shown.

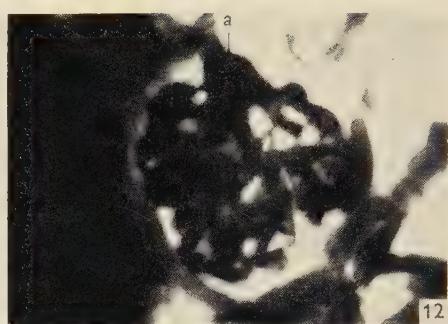
Fig. 15. Two 22 cm. foetal sheep glomeruli showing their general anastomotic arrangement; the right one shows a deep indentation of the peripheral channels producing lobulation.

Fig. 16. A 22 cm. foetal sheep glomerulus showing the anastomosing character of its vessels and surface lobulation.

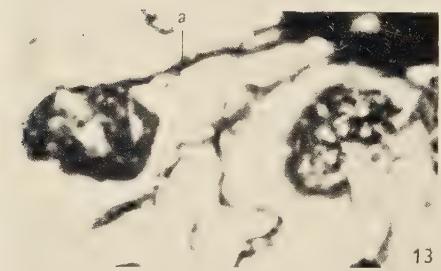




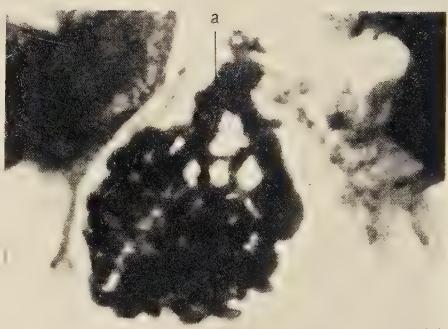
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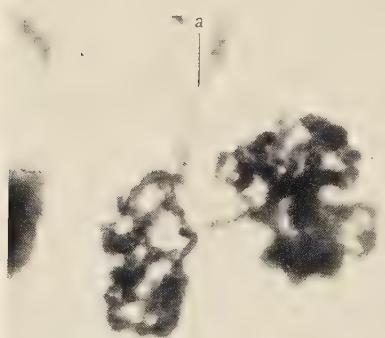
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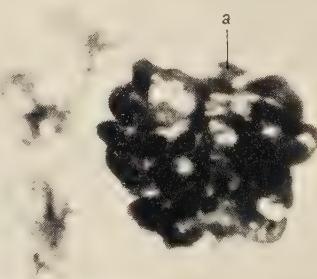
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HISTOLOGICAL OBSERVATIONS ON THE GASTRO-OESOPHAGEAL JUNCTION IN THE RABBIT

By G. S. MULLER BOTHA

Lecturer in Surgery, University of Birmingham

Basslinger (1860) first described the remarkable rhythmical activity at the gastro-oesophageal junction of the rabbit, by studying the isolated organs immediately after death. This unique, pulsating movement, better known as 'Basslinger's Pulse', has since been described by many other workers (Kronecker & Meltzer, 1883; Openchowski, 1883, 1889; Meltzer, 1897; Sinnhuber, 1903; Payne & Poulten, 1927). Recently the physiological activity of the entire oesophagus and cardia in anaesthetized animals has been studied in detail (Botha, 1958a). The literature offers no satisfactory explanation for this extraordinary phenomenon. As a local mechanism was considered to be the most likely cause for this dynamic activity, histological sections of the gastro-oesophageal segment in rabbits were studied. It is the object of this paper to report the outstanding features at the 'cardia' of the rabbit: a massive anatomical sphincter that consists of two elements—a striated as well as a smooth muscle bundle.

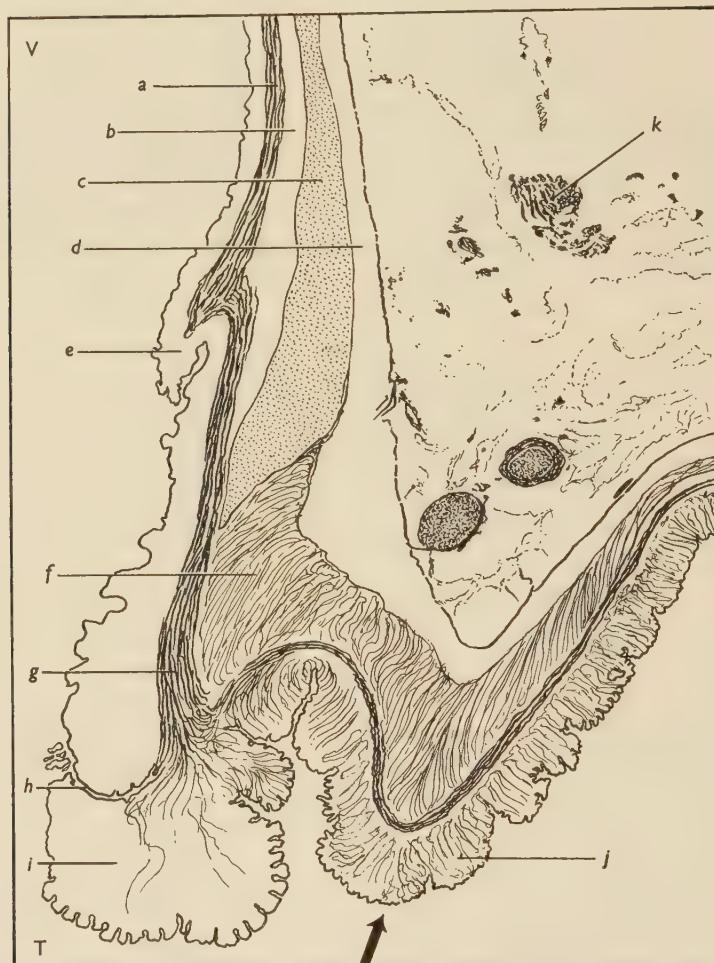
METHOD

I have pointed out that the macroscopical appearances during life or immediately after death present a striking difference from that which is seen a few hours after death (Botha, 1958b). The sphincter, on histological examination, was always however quite obvious. In order to obtain histological sections with the tissues and organs in the normal functional relationship, the stomach and lower oesophagus were removed immediately after death and fixed in 5% formol-saline. Sections were stained with haematoxylin and eosin; Van Gieson; Papanicolaou and Mallory's phosphotungstic acid haematoxylin. Pl. 1 shows a longitudinal section through the gastro-oesophageal junction. A line drawing (Text-fig. 1) simplifies the macro-photograph and should be referred to when the description is read.

NAKED EYE APPEARANCE

The oesophageal wall is thicker than the gastric wall; the thickness in the oesophagus is mainly due to the powerful muscular coat, whereas in the stomach it is due to the mucosa—the muscle being very thin. The cardiac incisura is well marked. The lower oesophageal wall or 'vestibule' is triangular shaped as a result of massive thickening of the muscle layers to four times the thickness of the oesophageal coat. Two distinct muscle bundles can be differentiated: a proximal, fusiform, closely packed, darker stained column and a more distal, irregular, loosely arranged, lighter stained mass. These two bundles can be distinguished quite well with the phosphotungstic acid haematoxylin stain. The former bundle appears bluish and the latter brownish red.

A coarse squamous fold occurs where the vestibule commences. A voluminous pedicled fold occurs at the cardiac orifice where atypical glandular mucosa meets squamous epithelium.



Text fig. 1. Drawing of section illustrated in Pl. 1. *a*, muscularis mucosa above the vestibule; *b*, inner longitudinal striated layer; *c*, middle circular striated layer; *d*, outer longitudinal striated layer; *e*, stratified squamous epithelium; *f*, circular smooth muscle layer; *g*, muscularis mucosa is well developed and splayes out to the mucosal fold; *h*, squamo-glandular junction; *i*, zone of 'cardiac glands'; *j*, normal gastric mucosa; *k*, diaphragmatic muscle. *T*, The terminal fold which forms the rosette. *V-T*, Vestibule. The arrow indicates the true cardia—at the level of the incisura.

HISTOLOGICAL APPEARANCE

Adventitia

The lower oesophagus up to just above the vestibule is covered by a thin serosal layer that lies immediately on the outer muscle coat. The only bare area is a narrow dorsal strip where the meso-oesophagus gains attachment. At the vestibule, the

adventitious connective covering becomes more noticeable, while the pleura is suddenly separated from the oesophagus by large fatty pads, which completely fill the cardiac incisura. In the fresh specimen these fatty-areolar pads must be removed before the cardia can be visualized adequately. This might be a reason why authors have failed to observe this obvious anatomical feature. Scanty elastic fibrils are present, but near the cardia and proximal stomach wall the elastic fibres are grouped together in bundles and are of a coarser nature. These fibres represent the phreno-oesophageal membrane found in other animals (Botha, 1958c), but are too indefinite here to be regarded as a definite structure. The elastic fibres penetrate the muscle coat and fuse with the connective tissue septa that divide the muscle bundles. The peritoneum covers the proximal stomach and is reflected on to the tendinous crura. There is no muscular attachment to the gullet.

The muscle

The muscular coat of the lower oesophagus consists of three striated layers. The middle circular layer is the most powerful. It is thick, well developed and tightly woven. The inner longitudinal layer is about half as thick as the circular layer, and is separated loosely from it by some connective tissue strands. The outer longitudinal layer is very thin; only two or three fibres thick. These fibres are wavy, interrupted, and are embedded in loose fibro-areolar connective tissue, rich in blood vessels. This layer is loosely attached to the circular layer and even individual fibres appear to be isolated by connective tissue fibres. 2–4 mm. above the vestibule all three layers increase in thickness.

The inner layer

Just above the vestibule the appearance of the muscle fibres changes. They become loosely packed, wavy and bigger (longer and broader); the cells appear swollen and hypertrophied. This dense fusiform mass gradually tapers to a thin elongated end distal to the proximal coarse fold. Instead of lying loose from the circular layer as in the oesophagus, it now emerges imperceptibly into this bundle so that the fibres can only be distinguished by the change in direction. The inner surface of this layer is closely fixed to the submucosal connective tissue.

The circular layer

The sphincteric thickening of the circular coat is most striking. This layer widens to a drawn-out mass, three times as thick as before. The large, thick, tightly packed fibres are divided into small compartments by numerous fibrous septa. This layer is closely adherent to and merges into the inner and outer layers. The dense ovoid mass is so well defined that its edges are quite distinct on the macro-photograph (Text-fig. 1), and it is almost entirely made up of striated muscle. Midway down the vestibule, this knob-like mass tapers to a sharp point.

The outer layer

This layer increases to about four times its former size, becoming thicker than the inner layer. Just above the cardia it is as thick as the circular layer, but the bundles are loosely packed and more irregular. The connective tissue septa are more prolific. Isolated smooth fibres appear where the vestibule commences and midway down,

almost half the fibres are smooth. Only isolated striated fibres are present at the cardia and then only in the longitudinal layer. A thin layer of longitudinal smooth muscle continues on to the gastric wall.

The smooth circular layer

The smooth circular layer commences just above the cardia as a roundish, irregular mass which is separated from the striated bundle by a definite connective tissue partition. Striated fibres are still abundant immediately distal to this zone, but soon disappear. This boundary is quite narrow so that the bulk of the muscle mass is entirely composed of smooth muscle. The fibres are not so closely packed as in the striated bundle and the columns of fibres are loosely arranged. The septa are numerous and of a looser texture. This muscle mass gradually decreases in size to its normal thickness a few mm. from the cardia. The muscle coat of the stomach is only half as thick as that of the oesophagus.

The submucosa

This is a very narrow zone of dense connective tissue. No superficial or deep oesophageal glands are present. The smooth muscularis mucosa is well developed, especially at the vestibule. Towards the terminal fold, the muscularis mucosa becomes a strong thick layer with large, long, smooth muscle fibres. At the base of the fold these fibres arrange in bundles and radiate towards the periphery of the fold. Terminal muscle fibres can be seen penetrating the atypical cardiac glandular epithelium and run almost to the surface. Numerous elastic fibres are present up to the submucosa of the terminal fold.

Mucosa

The thin layer of stratified squamous epithelium that lines the lower oesophagus increases in depth at the vestibule and is raised in horny transverse ridges towards the distal fold. The change from squamous to glandular epithelium is quite sudden—just proximal to the terminal fold. The basal layer of the squamous epithelium, immediately above the mucosal change, is irregular and broken up where submucosal connective tissue strands and muscle fibres of the muscularis mucosa gain firm attachment to the epithelial lining.

The mucosal covering of the terminal fold consists of atypical gastric epithelium and represents the zone of 'cardiac glands'. The glands are scanty, very tortuous and superficial. Near the surface the glands take up the stain well, but deeper in they are pale staining and probably mucus secreting. Parietal cells are absent or very scanty—a marked contrast to the abundance of these cells in normal gastric mucosa. On the distal border of the fold there is a sudden transition to normal gastric mucosa with long straight tubular glands and chief and parietal cells.

DISCUSSION

There can be no doubt that the rabbit presents a most remarkable anatomical sphincter at the gastro-oesophageal junction. The macroscopical appearances were uniform in more than forty consecutive rabbits. The histological appearances were constant in numerous sections of the cardia in specimens from five rabbits.

Anatomical sphincters have also been demonstrated in other animals (Botha, 1958c). In the bat the sphincter is composed entirely of smooth muscle; in the mouse of striated muscle. In the rabbit, however, this tremendously powerful muscle mass consists of both smooth as well as striated muscle. This is the only animal in which I have so far demonstrated a mixed sphincter. The level of the sphincter varies in different animals. In the bat it forms a ring at the cardia, whereas in the rat it is found cranial to the cardia. Even in other animal species and in man where anatomical sphincters have not been demonstrated, physiological sphincteric mechanisms do occur (Botha, 1958d; Botha, Astley & Carré, 1957).

Although the sphincter is located cranial to the cardia in the rabbit, the cardiac orifice is guarded by a pedicled fold of mucous membrane. This circular prolapsing papilla produces a watertight seal at the cardia and plugs the orifice as effectively as a cork in a bottle. This fold is not a passive, mechanical structure, but actively controlled by the inherent tone in the muscularis mucosa and its attachments to the sphincter. Together, the sphincter and the fold act synergistically to form the most wonderful closing mechanism between stomach and oesophagus that I have seen. It is also responsible for the amazing functional activity which aroused such interest in the past and which I have fully dealt with elsewhere (Botha, 1958e).

SUMMARY

A macroscopical study of the gastro-oesophageal junction in forty consecutive rabbits showed the presence of a massive muscular sphincter in the lower oesophagus and a serrated mucosal rosette at the cardiac orifice. Together, these two factors form a perfect closing mechanism between stomach and oesophagus.

Histologically the sphincter is composed of both smooth and striated muscle masses. This extraordinary feature is primarily responsible for the remarkable physiological activity at the rabbit's cardia.

I wish to thank Prof. F. A. R. Stammers for his constant encouragement; Dr W. C. O. Hill, of the Zoological Society of London, for his valuable help and advice in preparing this paper; Mr T. F. Dee for the illustration (Text-fig. 1); and the Endowment Fund for a grant.

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EXPLANATION OF PLATE

Longitudinal section through the gastro-oesophageal junction of the rabbit ($\times 15$, H. and E.).



MULLER BOTHA—GASTRO-OESOPHAGEAL JUNCTION IN THE RABBIT

(Facing p. 446)

THE CONTENT AND DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE BILIARY TRACT OF THE SHEEP

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INTRODUCTION

The histochemical distribution of alkaline phosphatase in the liver and gall bladder of the dog, rabbit and guinea-pig was studied by Jacoby & Martin (1951). These investigators showed that there was a rough correlation between the extent of the histochemical reaction of the bile caniculi in the liver and the enzyme level in the bile of the rabbit and dog, and that there was no reaction in the bile caniculi of the guinea-pig and virtually no enzyme in the bile of that species.

Before investigating certain aspects of liver metabolism in the sheep it was necessary to know the normal distribution of alkaline phosphatase in the biliary tract of this species, and in the absence of information in the literature the following study was undertaken.

MATERIALS AND METHODS

Healthy Clun Forest sheep from the Institute flock were used. The animals were brought in from pasture, stunned with a captive bolt pistol and bled out from the jugular vein. A sample of this blood was collected and the alkaline phosphatase content of the separated serum determined by the method of King & Armstrong (1934) using an E.E.L. photoelectric colorimeter. Next the abdomen was opened and the liver, gall bladder, bile ducts, pancreas and a portion of the duodenum were removed from the carcase as a unit. The liver of the sheep consists of a large main lobe partially subdivided by the umbilical fissure, and a smaller caudate lobe attached close to the renal impression. The long narrow gall bladder lies on the visceral surface close to the umbilical fissure and gives off the cystic duct which joins the hepatic duct to form a common bile duct. The latter is joined by the pancreatic duct on its way to the duodenum which is entered about 10 in. from the pylorus. The duct traverses the duodenal wall between the longitudinal muscle layer and the muscularis mucosae for about 2 in. in a caudal direction before entering the lumen of the intestine through a narrow slit in the mucosa.

Small portions of liver (from caudate lobe), gall-bladder wall, cystic duct, hepatic duct, common bile duct before the junction with the pancreatic duct, and of the common bile duct embedded in the duodenal wall were placed in formol-saline and in cold acetone. Kidney tissue was usually included as a positive control as indicated in Table 1. Bile collected from the gall bladder was centrifuged and its alkaline phosphatase content determined by the method of King & Armstrong (1934).

A total of eleven sheep was used, ranging in age from 35 weeks down to a foetus of about 16 weeks' gestation which had been removed by Caesarian section.

Portions of liver and kidney (about 20–40 mg.) were accurately weighed and homogenized with 2 ml. 0·9% saline in a tissue-grinder. The enzyme content of this homogenate was estimated and the results expressed as King-Armstrong units (K.A.) per gram of moist tissue.

In order to extend the observation of Jacoby & Martin (1951) to the extra hepatic tissues of the guinea-pig, two animals aged 20 and 26 weeks were also used. They were stunned by a blow on the head, bled from the neck vessels and material was collected in the same manner as from the sheep.

The formalin fixed material, sectioned and stained with haematoxylin and eosin, was examined for general morphology.

The acetone-fixed material was processed by a modification of Gomori's (1939) method after fixing at 4° C. for 24 hr. Two 12 hr. changes of absolute acetone were followed by alcohol/ether and then 0·5% celloidin for 24 hr. After $\frac{1}{2}$ hr. in chloroform the tissue was embedded in paraffin wax at 50° C., blocked, and sections 5–6 μ thick were cut. The sections were mounted on clean slides, dried at 50° C. for 10 min., dewaxed with xylol, taken into absolute alcohol and covered with 0·5% celloidin.

The sections were then incubated at 37° C. for 4 hr. in the following mixture:

3% (w/v) sodium glycerophosphate	10 ml.
2% (w/v) calcium chloride	25 ml.
10% (w/v) magnesium sulphate	0·5 ml.
Sodium barbitone	1 g.
Distilled water to	50 ml.

After washing under the tap for 1 min. the sections were treated with 2% cobalt nitrate for 5 min., washed again, and immersed in a jar of distilled water containing a few drops of ammonium sulphide. After a final washing in tap water for 3 min. the sections were dehydrated, cleaned and mounted in D.P.X.

Following microscopical examination the extent of the alkaline phosphatase reaction was given a rough quantitative assessment. The scoring was not based on the intensity of the deposits but rather on their extent. A liver score (+) would indicate staining of the bile caniculi in scattered locations, whilst (++) indicates general staining of caniculi throughout the section. In the extrahepatic biliary system scattered staining of the tunica propria merited (+), whilst continuous staining of this area in the wall of the gall bladder or duct was scored (++) .

RESULTS

Sheep. Examination of the haematoxylin- and eosin-stained sections confirms that the microscopical structure of the biliary tract of the sheep is of the usual mammalian type, as described by earlier anatomists, e.g. Oppel (1896). The bile canaliculi between the hepatic cells drain into bile duets in the portal tracts. These bile duets are lined by a single layer of cubical cells and anastomose to form the hepatic duct which emerges on the visceral surface of the liver. This duct, the cystic duct, the gall bladder and the common bile duct, are lined by a simple columnar epithelium folded to varying degrees and carrying numerous mucous cells. Mucus secreting glands are buried in the connective tissue and their number increases as the duodenum is

approached. Where the duct opens into the lumen of the duodenum, the lining epithelium is continuous with the columnar epithelium of the duodenum.

A point of interest in the biliary tract of the sheep is the presence in the epithelium of the bile ducts and their accompanying mucous glands of numerous 'globule leucocytes' (Keasbey, 1923; Kent, 1952). These cells are absent from the biliary tract of the foetus and the 1½- and 2-week-old lambs, but increase in frequency with the age of the sheep examined. Sommerville (1956) has noted that the presence of these cells in the abomasal mucosa of sheep is associated with parasitic infestation, an association which would also be feasible for the sheep of this series as it is well known that young sheep on pasture invariably carry a number of gastro-intestinal helminths.

The results of the examination of the sheep and guinea-pig material for alkaline phosphatase are summarized in Tables 1 and 2, respectively.

Table 1. Alkaline phosphatase values in the serum, bile, liver, kidney and bile ducts of ten sheep

Sheep No.	Age (weeks)	Alkaline phosphatase content K.A. units/g. or /100 ml.				Histochemical findings						
		Liver		Gall		Cystic	Hepatic	Common	Duct in	Kidney		
		Kidney	Liver	Bile	Serum	bile canaliculi	bladder	duct	duct	intestinal wall	tubules	
Foetus 1	-4	-	11	39	-	0	+++	0	0	-	-	-
J 12	1½	44	9	145	100	+++	+++	+	0	0	+	++
H 183	2	-	-	-	427	+++	++	+	0	0	++	-
H 169	4	44	-	-	-	++	+++	0	0	-	-	++
J 183	4½	58	8	194	54	+++	+++	++	++	+	++	++
H 176	6	70	33	440	53	+++	+	0	0	+	++	+++
H 65	13	-	-	164	16	+++	++	+	++	0	++	++
H 38	16	100	14	62	-	+++	++	0	0	0	++	++
H 25	20	23	22	88	24	+++	+++	++	+	+	++	++
H 43	24	30	7	63	14	+++	+++	++	0	+++	+++	+++
H 37	35	33	6	65	14	+++	+++	++	++	+++	+++	+++

+ = scattered areas of reaction; ++ = fairly extensive reaction; +++ = complete reaction of liver canaliculi or of tunica propria; 0 = no reaction; - = no specimen.

Table 2. Alkaline phosphatase values in the serum, bile, liver, kidney and bile ducts of two guinea-pigs

Guinea-pig No.	Age (weeks)	Alkaline phosphatase content K.A. units/g. or /100 ml.				Histochemical findings					
		Liver		Gall		Common		Kidney			
		Kidney	Liver	Bile	Serum	bile canaliculi	bladder	bile duct	tubules		
GP1	26	100	2.6	0.4	8	0	0	0	0	+++	
GP2	20	77	3.5	0.7	8	0	0	0	0	++	

+++ = intense staining of tubules; 0 = no alkaline phosphatase staining.

Table 1 shows that the alkaline phosphatase content of the gall bladder bile of ten sheep and one foetus varied from 39 to 440 K.A. units/100 ml. of bile, whilst serum levels of the enzyme varied from 14 to 427 K.A. units/100 ml. of serum. In the serum, high levels were found in the younger sheep, an observation not inconsistent with that of Allcroft & Folley (1941). Bile levels of the enzyme rise to a peak at about 6 weeks of age, after which values fall to a lower level.

The liver bile canaliculi were generally stained throughout the lobule, whilst staining of the bile ducts within the peri-portal connective tissue was variable; in some cases

the tissue surrounding the cubical cells was stained, whilst in others the duct was quite negative.

In the extra-hepatic biliary system of the sheep staining reactions in the epithelium were consistently negative and deposits if present always occurred in the tunica propria below the epithelium. Some of the reacting material was localized in the walls of capillaries, but most of the deposit was distributed in the cytoplasm of the tunica cells. The sign (+ + +) indicates that in a cross-section of a duct the deposits occurred in a complete circle, whilst (+) indicates scattered localized deposits. It will be seen that the gall bladder and the duct passing through the intestinal wall were consistently positive, whilst the cystic, hepatic and common ducts varied in the extent of the deposits, in many cases staining being completely absent. Pl. 1, figs. 1-4 illustrate the enzyme deposits in the gall bladder and common bile ducts of two sheep, nos. J12 aged 1½ weeks and H37 aged 35 weeks. In both sheep the tunica propria of the gall bladder stained continuously, but the common bile duct reactions differed; J12 was negative and H37 stained strongly in the tunica propria.

One foetus was also examined. Table 1 shows that the bile alkaline phosphatase level was about half the adult level and only a fraction of that present in the young lambs. The bile canaliculi did not stain, the extra-hepatic ducts were negative, but the tunica propria of the gall bladder was completely stained.

Guinea-pig. The results given in Table 2 confirm the findings of Jacoby & Martin (1951) in respect of the lack of staining in the gall bladder and liver bile canaliculi and the low enzyme levels in bile. The table also indicates that the common bile duct shows no alkaline phosphatase staining, and that levels of the enzyme in liver tissue are appreciably lower than in the liver of the sheep at all ages. Guinea-pig kidney, on the other hand, has enzyme levels comparable to those of sheep kidney and the tubules stain equally extensively.

DISCUSSION

This investigation has shown that the alkaline phosphatase content of the bile and serum of the sheep varies considerably. There is a marked histochemical reaction for the enzyme in the bile canaliculi of the liver and in the tunica propria of the gall bladder, in the part of the bile duct within the duodenal wall and in the kidney tubules of sheep from 1½ to 35 weeks of age. The tunicae propriae of the cystic, hepatic and common ducts stain variably, in some animals the tunica propria is completely negative, whilst in other animals the tunica propria of these ducts stains extensively. Jacoby & Martin (1951) found a similar variation in the amount of the enzyme in the bile and bile canaliculi of the rabbit and dog.

It is generally held that, in most animals, alkaline phosphatase entering the serum from organs such as bone, kidney and intestine, is excreted by the bile and that the rise in serum alkaline phosphatase levels which follow natural or experimental biliary obstruction is due to interference with this excretion. According to Flood, Gutman & Gutman (1937) another channel of enzyme excretion in the cat is probably the urine, and Jacoby & Martin's failure to find the enzyme in the bile of the guinea-pig suggests a different channel of excretion in that species.

The sheep, however, in view of the high enzyme content of its bile, appears to fall into the same category as the rabbit, dog and man, and it appears that the determination of serum alkaline phosphatase levels would be a useful method of following any experimental liver lesions which might produce interference with bile excretion. This suggestion is supported by the failure to detect the enzyme in a number of samples of fresh urine from normal sheep. The variable occurrence of the enzyme in the tunica propria at different sites in the extrahepatic biliary tract is interesting. Jacoby & Martin (1951) suggest that the presence of the enzyme in the epithelial cells of the dog's gall bladder is associated with the absorption of fat from the bile by the epithelium, and discard the theory that the enzyme itself has been absorbed from the bile as the enzyme occurred in gall bladders of dogs with alkaline phosphatase-negative bile.

However, in the sheep the epithelial cells were never positive, as one might expect them to be if the enzyme took part in the absorption of material from the bile. For the same reason it is also unlikely that the deposits in the tunica propria have been absorbed from the bile. In fact, in view of the variable extent of the enzyme staining in individual sheep it is difficult to suggest a valid reason for its presence in the tunica propria of the gall bladder and extrahepatic ducts.

It is interesting to note that in the common bile duct passing through the intestinal wall the enzyme constantly occurs only in the tunica propria, yet the cells of the intestinal epithelium are positive only at their brush borders. In view of the continuous nature of the epithelia of the duct and of the intestine there obviously must be a point at which one type of staining changes to the other, but despite the examination of numerous sections from the region of the bile duct orifice, such a point was not detected.

SUMMARY

1. The hepatic and extra-hepatic biliary tracts of ten sheep from $1\frac{1}{2}$ to 35 weeks of age, of one foetus of about 16 weeks' gestation and two guinea-pigs have been examined for the presence of alkaline phosphatase by a histochemical technique (Gomori, 1939).

2. The alkaline phosphatase content of bladder bile, blood serum and liver tissue has been examined by the method of King & Armstrong (1934). Kidney was examined by both methods as a positive control.

3. The enzyme levels in bile and serum are high in the young lamb and the occurrence of the enzyme in the tunica propria of the common, hepatic and cystic ducts, as demonstrated by a histochemical technique, is very variable.

4. The tunicae propriae of the duct in the intestinal wall, of the gall bladder and the bile canaliculi of the liver are positive at all ages, but in the foetus only the tunica propria of the gall bladder is positive.

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EXPLANATION OF PLATE

Fig. 1. Lamb J12. Age 1½ weeks. Gall bladder. Gomori $\times 80$.

Fig. 2. Lamb J12. Common bile duct. Gomori $\times 80$.

Fig. 3. Sheep H37. Age 35 weeks. Gall bladder. Gomori $\times 80$.

Fig. 4. Sheep H37. Common bile duct. Gomori $\times 80$.



1



2



3



4

FORD—CONTENT AND DISTRIBUTION OF ALKALINE PHOSPHATASE

(Facing p. 452)

A HISTOCHEMICAL STUDY OF THE STOMACH AND INTESTINE OF THE CHICKEN

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INTRODUCTION

The literature concerning the microscopic anatomy of the digestive tract of the domestic fowl is voluminous, and a comprehensive review of this work with numerous excellent photomicrographs has been published recently (Calhoun, 1954). Several features, however, require further investigation. Although it has been shown that the contents of both proventriculus and gizzard are acid (Browne, 1922; Vonk, Brink & Postina, 1949), the location of acid-producing cells has not been conclusively determined. Browne (1922) states that the submucosal glands of the proventriculus secrete acid gastric juice but offers no evidence in support of this statement, whilst Bradley & Grahame (1950), on the contrary, state that there are no parietal (oxytic) cells in the proventriculus. Batt (1924), although claiming that the submucosal gland cells do resemble the acid-producing cells of the mammalian stomach, does not detail points of resemblance and in any event appears to think that the resemblance is slight.

The information relating to pepsin-producing cells is even less specific than that relating to acid production, although Vonk *et al.* (1949) have demonstrated peptic activity in gizzard contents.

Few attempts have been made to characterize chemically the cuticular layer of the gizzard, although the majority of authors refer to it as 'horny' and appear to consider that it is a keratinous substance. Bradley & Grahame (1950), on the other hand, consider this material to be keratohyalin, but do not indicate on which reactions this claim is based. Calhoun (1954) also considers the horny material to be keratohyalin, basing this claim on the results of a single reaction. This horny material is produced by gland cells which appear to be epicrine in their mode of secretion and therefore its formation is quite distinct from that of other keratinous substances. In view of this and of the inconclusive nature of the information relating to the composition of this substance, further study is justified.

The morphology and distribution of argentaffin cells do not appear to have been studied extensively in the chicken. Dawson & Moyer (1948) found numerous argentophile cells in the proventriculus and gizzard, but were unable to demonstrate the presence of argentaffin cells in these organs; whilst Simard & van Campenhout (1932), in their study of the embryonic development of argentaffin cells in the chicken, found these cells in only one specimen of proventriculus and apparently found none in the gizzard. The comparative rarity of argentaffin cells in the stomach may be related to the structural peculiarities of this organ in the chicken and may prove helpful in elucidating problems associated with the function of argentaffin cells in general.

Simard & van Campenhout (1932) have described the development and distribution of argentaffin cells in the chicken and have suggested that at one stage in development (269 hr.) argentaffin cells migrate from the epithelium into the lamina propria. This migration does not appear to have been noted in other species. Schofield (1950, 1952, 1953) found that in the guinea-pig, mouse and man the argentaffin cells of the small and of the large intestine are functionally and morphologically distinct, those of the small intestine alone being involved in a secretory cycle culminating in the formation of a mucous goblet cell. The possibility that argentaffin cells at different gut levels may subserve different functions is obviously of great interest and demands further investigation, particularly from a comparative point of view. This author also states that the number of silver 'staining' granules in the intestinal argentaffin cells is greatest where the staining method used involves the application of an extraneous reducing agent, i.e. only some of the granules are argentaffin whilst some are argentophile. This lends support to the view expressed by Dawson & Moyer (1948) that argentaffin cells are at first argentophile, and is of particular interest in view of possible differences in function of argentaffin and argentophile cells.

MATERIALS AND METHODS

The specimens described hereafter were obtained from nine White Leghorns ranging in age from 6 weeks to 2 years, distributed as follows: 2 ♂ at 6 weeks, 1 ♀ at 10 weeks, 3 ♂ at 5 months, 3 ♀ at 18–24 months.

In each case tissue was taken from proventriculus, gizzard, gizzard-duodenal junction, caecum and large intestine. In some cases tissue was taken also from proventriculus-gizzard junction, the terminal part of the small intestine, and from the coprodaeum.

Tissue from each locality was fixed in buffered neutral formol and in Orth's fluid. Sections from all blocks were routinely stained with haematoxylin and eosin, trichrome stain (Gomori, 1950a), aldehyde fuchsin (Gomori, 1950b), mucicarmine, methylene blue, celestin blue, PAS (Gomori, 1953). In addition in appropriate instances the following methods were employed: 1% thionin (pH 4–5—Lillie, 1954); azure A—eosin B (pH 3·75–5—Lillie, 1954); eosinophil myelin (Lillie, 1954); picocarmine; picric acid-nigrosin; ferric ferricyanide reduction for SH groups (Lillie, 1954); azure A—eosin B following oxidation by peracetic acid (Lillie, 1954); Best's carmine; Sudan black B; Gomori's methenamine-silver (alone and combined with mucicarmine); Bodian's protargol method (original method and as modified by Dawson & Barnett, 1944); azo-coupling and indophenol reactions (Gomori, 1953).

RESULTS

Proventriculus

The mucosa of the proventriculus contains simple tubular glands lined throughout their length by a columnar epithelium the cells of which diminish in height towards the blind end of the tubule. Most of the cells of this epithelium contain granules of mucin, but these are much less numerous in the cells lining the blind end of the tubule where in fact they are sometimes entirely lacking; towards the open end of

the tubule they may again diminish and are sometimes absent from the lining epithelium of the proventriculus.

The submucosal glands of the proventriculus are arranged in lobules, each consisting of numerous uncoiled tubules radiating from a central cavity into which they discharge. Except near their open ends these tubules are lined by a simple epithelium consisting of cells which only make contact with adjacent cells towards their bases and consequently the epithelium has a dentate appearance (Pl. 1, fig. 1). Towards the open end of the tubule, however, the epithelium is composed of tall, columnar cells which are closely applied to one another and therefore do not exhibit this dentate appearance. A similar epithelium lines the central cavity. The latter is drained by a duct with an irregular lumen with tubular evaginations extending into the adjacent tissue. This duct leads through the mucosa to the free surface.

The epithelial cells lining the main excretory duct and the central cavity exhibit staining reactions identical with those characteristic of the epithelium of the mucosal glands, but as the distance from the mucosa increases, the number of secretory granules in the cells diminishes and they may be completely absent from the excretory ducts of individual gland tubules.

In sections stained with Gomori's trichrome, however, red granules are found scattered throughout the cytoplasm of the secretory cells of the submucosal glands, often appearing in similar concentration in both the basal and luminal cytoplasm, although when particularly abundant they tend to accumulate in the luminal cytoplasm (Pl. 1, fig. 2). The granules are large, rounded and are reddish in colour, though variations in staining reaction are noted in different cells, some showing a particularly intense red. Cells showing this intense reaction may form the entire lining of a particular gland tubule or they may be scattered singly or in small groups amongst less intensely stained cells. Fixation in buffered neutral formalin does not affect the degree of granulation which is then just as distinct as in preparations fixed in Orth's fluid.

In sections stained by methylene blue or thionin at pH 4-5 these cytoplasmic granules remain unstained and therefore the cells have a vacuolated or foamy appearance. The intervening cytoplasm exhibits varying degrees of basophilia and in the more intensely stained preparations may give the cell a finely granular appearance, although it is reasonably certain that the cells do not contain any granules other than those mentioned above. The reaction to pyronin paralleled that noted in the methylene blue and thionin preparations. In no case, however, was the basophilia or pyronin reaction intense.

In preparations stained with azure A-eosin B the cytoplasm was coloured light blue whilst the granules were either unstained or stained a very faint pink colour. No variations in staining reaction were noted between Orth fixed and formalin fixed preparations or as a result of varying the pH from approximately 3.75 to 5.

Argentaffin cells occur very infrequently in the mucosa and are entirely absent from most sections. They are not present in the submucosal glands.

Gizzard

In the gizzard the glands are simple uncoiled tubules lined throughout the greater part of their length by low cuboidal cells. Towards the open end of the tubule

however the cells become taller and in the upper part of the glands and over the free surface are columnar. The gland lumen is frequently filled with a secretion which accumulates on the mucosal surface of the gizzard to form a thick layer of 'horny' material (Pl. 1, fig. 3).

The columnar cells covering the free surface and lining the upper part of the gland tubule contain granules which stain with mucicarmine, PAS and aldehyde fuchsin and which are metachromatic with celestin blue and methylene blue (Pl. 1, fig. 4). In some instances the secreted material at the mouths of the glands exhibits similar reactions and the secretion which accumulates on the surface of the mucosa occasionally exhibits a horizontal striation, due to layers of material which give the above reactions alternating with layers of non-reactive substance (Pl. 1, fig. 3).

The cuboidal cells which line the greater part of the gland tubule on the other hand do not give the above-mentioned reactions. In preparations stained by the Gomori trichrome technique these cells are found to contain very fine red granules which are concentrated particularly in the luminal cytoplasm. These are not readily distinguished in sections stained with Lillie's eosinophil myelin stain but may sometimes be seen and are then dark blue or brown in colour. They remain uncoloured in sections stained with Delafield's haematoxylin, azure A-eosin B, picrocarmine, Sudan black B, PAS or picric acid-nigrosin, and are not argentophile.

The secreted material within the gland tubules and covering the free surface is also coloured red by the Gomori trichrome technique and a very dark blue or dark brown with Lillie's eosinophil myelin stain, both colours in some instances being present in the same section. This material is PAS positive even after treatment with saliva, but the reaction is most pronounced in the gland tubules, the reaction of the material on the surface, although varying somewhat from section to section, being generally much weaker and occasionally negative except for vertical streaks opposite the mouths of the glands (Pl. 1, fig. 3). A similar, though perhaps slightly less intense reaction, is noted on staining with Schiff without pretreatment with periodic acid. (Sections of mammalian skin stained simultaneously with sections of gizzard give negative results with the PAS technique both with regard to hair cortex and stratum corneum.) The secretion, both on the surface and in the gland tubules, is coloured yellow in sections stained with picric acid-nigrosin or picrocarmine. In sections stained with azure eosin or thionin following oxidation with peracetic acid, the secretion is generally coloured varying shades of blue, the material within the gland lumen generally being a dark blue whilst that on the surface is a light-greenish blue streaked perpendicularly with lines of a darker blue corresponding to the mouths of the glands (Pl. 2, fig. 5). (Both these colours may be observed in mammalian hair cortex at hypodermal levels when stained by this method.) In some instances, however, the secretion in the lumen of some of the glands in a section stains a very faint pinkish tinge. This is not comparable to the red characteristic of stratum granulosum or stratum corneum of mammalian skin, in both of which a much more distinctive red is obtained on staining by this method.

Neither the gland cells nor the secreted material give a positive reaction with the ferric ferricyanide test for sulphhydryl groups except at the free surface of the secretion mass lying on the mucosa where a line of reactive substance may be noted.

Variable amounts of pyronin-positive material are found in the cuboidal cells lining the gland tubule, this material extending throughout the greater part of the cytoplasm in some cells, whereas in others it is present only in small quantity and sometimes is completely absent. When present, it tends to be concentrated particularly in the basal cytoplasm.

Argentaffin cells appear to be absent from the gizzard.

Gizzard-duodenal junction

Between the gizzard and the duodenum there is a transitional zone measuring approximately 0·5 cm in length. This zone is readily distinguished from the gizzard by the villous character of the mucosa and by some degree of coiling of the glands which are lined by an epithelium which is composed of tall columnar cells and therefore unlike that of the glands of the gizzard proper. Intermingled with the columnar cells are large rounded cells the cytoplasm of which is usually unstained or lightly stained in most preparations.

On the other hand, this transitional zone is readily distinguished from the duodenum by the fact that all the epithelial cells covering the surface of the villi and the free surface of the mucosa are mucus secreting, whereas in the duodenum proper this function is restricted to goblet cells scattered singly amongst columnar cells with a striated border. The latter are absent from the junctional zone. In the duodenum, as in all parts of the intestine, the lower parts of the gland tubules are lined by mucus secreting cells, thus further distinguishing the duodenum from the transitional zone in which a mucus secreting function is largely confined to the superficial cells and to the upper parts of the gland, the number of PAS positive granules diminishing rapidly as the distance from the surface increases.

A further feature by which the transitional zone is distinguished from the duodenum is that argentaffin cells are absent except in its terminal part immediately adjacent to the duodenum where in some specimens argentaffin cells may be found. They are much less numerous in this locality, however, than in the immediately succeeding section or the duodenum where they are more numerous than anywhere else in the gastro-intestinal tract (Pl. 2, fig. 7).

In sections stained by the Gomori trichrome technique, it is possible to distinguish granular and agranular cells in the glandular epithelium. In some cases the granular cells are identical with the large poorly staining cells mentioned above, but this is not constantly the case. The granules are very fine and are not a prominent feature. They remain unstained in sections stained with thionin at pH 4–5 and in azure A–eosin B preparations.

In the transitional zone the ‘keratinous’ layer which covers the mucosa of the gizzard gives way to a layer of mucus of similar thickness. This stops abruptly at the beginning of the duodenum.

Glands resembling mammalian Brunner’s glands are not present in the transitional zone nor in the adjacent part of the duodenum.

Intestine

In both small and large intestine the surface epithelium is simple columnar and consists of goblet cells scattered amongst columnar cells with a striated border

(Pl. 2, fig. 8), the former apparently increasing in number as the gut is traced caudally. The glands are simple tubules slightly coiled and are lined by stratified or pseudo stratified columnar epithelium.

The goblet cells and all the cells lining the blind ends of the glands contain granules of mucin. Brunner's glands are apparently lacking and Paneth cells are not present in either small or large intestine; eosinophil leucocytes also appear to be absent from the intestinal mucosa.

Argentaffin cells are present at all levels of the intestine including the coprodaeum. They are most numerous in the duodenum in a narrow zone at its origin, a feature particularly marked in younger birds in which this zone is very narrow and is succeeded by one in which lymphatic tissue is particularly abundant and gland tubules in consequence are widely separated from one another. In the latter zone the number of argentaffin cells per field is greatly reduced. This reduction, however, is not accounted for entirely by the reduction in the number of gland tubules, there being also a reduction in the number of cells per tubule. In older birds the amount of lymphatic tissue in this area is considerably less. Nevertheless, here also there is a reduction in the number of cells per field although this is not so spectacular as in the younger birds, in which in the abundant zone there may be as many as 26 cells per field, whereas in the adjoining zone the number falls to some 4–6·8 per field. (The term 'field' implies, in this instance, a microscopic field using a $\times 45$ fluorite oil-immersion objective and a $\times 10$ eyepiece.) Thereafter the number of argentaffin cells per field is fairly constant throughout the small intestine. Argentaffin cells are also found in the caecum and large intestine and are more abundant in the latter than in the small intestine other than in the area immediately following the transitional zone.

The argentaffin cells may be located mainly in the blind ends of the glands but sometimes are found to be equally numerous at all mucosal levels, this being particularly a feature of younger birds (Pl. 2, fig. 9). These cells are invariably located in the epithelium and never in the connective tissue and usually are in contact with the basement membrane, although occasionally cells may be found apparently migrating towards the free surface (Pl. 3, fig. 10). Rarely the argentaffin cells are arranged in small groups of two to three contiguous cells.

The number of argentaffin granules per cell varies widely. In a few only a small number of granules are present and are then mainly located in the basal cytoplasm close to the basement membrane (Pl. 3, fig. 11), or very occasionally are arranged as a halo around the nucleus, but generally the granules are much more abundant than this, frequently completely filling the basal cytoplasm, obscuring the nucleus and usually extending to a variable extent into the luminal cytoplasm. In the latter case the cell may be extended towards the lumen of the gland or surface of the intestine in the form of a slender process containing a few discrete granules or, more rarely, as a thicker process filled with densely packed argentaffin material in which the individual granules can rarely be distinguished (Pl. 3, fig. 12). Intermediate forms between these extreme types are also found which are regularly triangular with the base resting on the basement membrane and the apex directed towards the free surface which it may reach. In this type the granules in the luminal cytoplasm are abundant but discrete (Pl. 3, figs. 13, 14). The nucleus is generally

located in the cytoplasm near the basal surface, but in occasional cells it is displaced towards the free surface and in these the extended basal cytoplasm is so deeply stained that individual granules cannot be distinguished (Pl. 3, fig. 14). In occasional cells the basal cytoplasm is extended laterally and these therefore are wider than average at this point. Occasionally this extension appears as a short process running laterally for a short distance between the overlying cells, and the basement membrane. Very rarely the basal cytoplasm is vacuolated (Pl. 3, fig. 13), and even more rarely intracellular fibrils appear to be present. It is possible, however, that the latter appearance is due to linearly disposed granules, the concentration of the latter in cells in which this appearance is noted being too great to permit accurate observation.

Every type of argentaffin cell found in the small intestine is duplicated in the large intestine and the reverse is also true. Furthermore, no differences in cell types or in the number or disposition of argentaffin granules in individual cells are noted between specimens stained by the methenamine silver technique (without subsequent reduction) and those stained by the modified Bodian technique with subsequent reduction.

The granules in chick argentaffin cells are very strongly eosinophilic, stain bright red with the Gomori trichrome stain, give diazo and indophenol reactions and are also positive with the ferric ferricyanide reduction test (Pl. 3, fig. 11). (This response was noted incidentally when examining sections of gizzard-duodenal junction for SH groups at the presumed site of keratin production in the gizzard. In such specimens the argentaffin granules in cells in the duodenum were strongly stained and almost as readily distinguished as in specimens stained by the silver techniques. This has been noted by Laskey & Greco, 1948 (quoted by Lillie, 1954).

In sections stained with Southgate's mucicarmine after hexamine silver the argentaffin cells remain uncoloured. This is true even of those cells in which the basal cytoplasm contains no argentaffin granules.

DISCUSSION

Proventriculus

Batt (1924) has stated that there are 'no marked acidic or pepsin-producing cells' in the submucosal glands of the proventriculus, but also claims that in their staining reactions the cells of these glands 'resemble somewhat the acid-producing or parietal cells of the mammalian stomach'. Browne (1922) states that the submucosal glands secrete acid gastric juice, but does not offer any evidence that the submucosal gland cells are in fact responsible for acid production. Vonk *et al.* (1949) found that the secretion of the proventriculus was acid, but that it was less so than the contents of the gizzard. Bradley & Grahame (1950), on the other hand, state that there are no parietal (oxytic) cells in the proventriculus.

A reaction similar to that noted in the submucosal gland cells in sections stained by the Gomori trichrome technique is noted also in the parietal cells in sections of mammalian stomach (dog, cat, rat and sheep) stained by this technique. This reaction, however, is not specific for hydrochloric acid secreting cells, and although in the circumstances the response is suggestive, the fact remains that the submucosal

gland cells do not exhibit the degree of eosinophilia noted in mammalian parietal cells. Whether the variations in the intensity of the reaction noted in the trichrome preparations indicate different functional states of the same cell type is uncertain, but this seems likely since similar variations are noted in parietal cells in mammalian stomach stained by this method.

The evidence with regard to the production of pepsin by the submucosal gland cells is more conclusive in that the failure to stain secretion granules with thionin at pH 4–5 strongly suggests that these cells are not zymogenic, and although occasionally a very faint pink colour is noted in the cytoplasmic granules in azure eosin preparations, this is of doubtful significance, particularly as it is not intensified on varying the pH of the staining fluid. Again, the basophilia in the submucosal gland cells is never pronounced, nor do these cells contain significant quantities of pyronin positive material in their basal cytoplasm as might be expected if they were zymogenic. These observations apply equally well to Orth-fixed and formalin-fixed preparations, and therefore it is unlikely that the failure to demonstrate pepsinogen granules is due to imperfect fixation. Furthermore, the cytoplasmic granules noted in trichrome preparations are equally prominent in formalin-fixed and Orth-fixed material which suggests that they are not pepsinogen since, at least in mammals, pepsinogen granules are poorly preserved after formalin fixation but well preserved in Orth-fixed tissue (Lillie, 1954).

Gizzard

Various authors have applied the term 'horny' to the secretion of the glands of the gizzard, but only Calhoun (1954) appears to have attempted to characterize this material on a chemical basis. Bradley & Grahame (1950) state that this substance is keratohyalin, but do not indicate which tests were employed to establish this claim. Calhoun (1954) also considered the horny substance to be keratohyalin and in sections stained by Pasini's method was able to demonstrate secretion granules in the adjacent cells. However, Opdyke (1952) states that the granules of keratohyalin in the stratum granulosum of stratified squamous epithelium stain beautifully with picrocarmine, have an intense affinity for all haematoxylin stains, stain metachromatically with toluidine blue and are argentophile, whereas the granules in the gland cells of the gizzard exhibit none of these reactions. In sections stained with Lillie's eosinophil myelin some staining of cytoplasmic granules does occur but their colour when they responded to this technique is indicative of keratin rather than keratohyalin (Lillie, 1954). Sections stained by this method are, however, difficult to interpret as far as cytoplasmic granules are concerned and this conclusion should be confirmed before it is accepted that the granules are keratinous, particularly as they do not respond to other stains known to stain keratin. Failure to stain with the azure A–eosin B technique indicates that the granules are not trichohyalin.

The secreted material in the gland lumina and on the free surface on the other hand gives many of the reactions characteristic of keratin. Thus it is stained yellow with picric acid–nigrosin and picrocarmine, and in sections stained with azure A–eosin B or thionin after oxidation with peracetic acid it gives reactions indicative of a substance containing cystine. The reaction obtained with the latter technique is similar in most cases to that of the hypodermal portion of mammalian hair root

which exhibits a peripheral dark blue portion and a central greenish blue part. In the gizzard the more darkly stained material is that within the gland lumen and extending as narrow vertical streaks from the mouths of the glands towards the free surface, and therefore the most recently formed secretion in the gizzard gives the most intense reaction whereas in hair the older free portion tends to be more intensely stained than the hypodermal part. The faint pinkish tinge noted occasionally with this technique does not correspond to the colour noted in keratinous structures in the epidermis in which a more decided red was generally characteristic of stratum granulosum (keratohyalin) and stratum corneum, although sometimes the outermost layers of the stratum corneum did give a somewhat similar reaction.

According to Lillie (1954) the keratin of stratum corneum yields a light to dark brown colour when stained by his eosinophil myelin, whilst hair cortex stains varying shades of blue. The bulk of the secreted material in the gizzard thus resembles hair cortex in yielding a dark blue colour when stained by this method. Occasionally, however, patches of brownish material suggestive of stratum corneum are present.

From the foregoing, therefore, it seems reasonable to conclude that the material secreted by the glands of the gizzard is a form of hard keratin and there appears to be little support for Calhoun's (1954) contention that its production is preceded by the formation of granules of keratohyalin. This material most closely resembles the keratin of hair in its staining reactions, but differs in some respects, particularly in its response to PAS or to Schiff without preliminary oxidation by periodic acid. Again, in their study of keratin production in epidermis and its derivatives Giroud & Leblond (1951) demonstrated the constant presence of sulphur as sulphydrol groups at the site of keratin production. The absence of such groups in the gizzard indicates that here the method of keratin production is different from that in skin and its derivatives, and this may be related to the fact that in the gizzard the keratinous material is discharged by the cells which are apparently epicrine in type.

The response to the Schiff reagent, even without preliminary oxidation with periodic acid, is not understood, but clearly it is not due to glycogen since it occurs even after treatment with diastase and is negative with Best's carmine. It is interesting, however, that a corresponding reaction is not noted in the gland cells and that it is much reduced in the secretion layer on the surface suggesting the possibility of a progressive chemical change beginning immediately after secretion.

The mucus secreted by the cells lining the gland tubules near the free surface and covering the latter may serve a protective or adhesive purpose. Horizontal streaks of faintly metachromatic material in the overlying keratinous mass suggest that this mucous secretion is gradually incorporated into the latter.

Gizzard-duodenal junction and intestine

Calhoun (1954), in her review of the literature relating to the intestine of the chick, states that 'all authors with the exception of Kaupp (1918) are agreed that Brunner's glands are lacking', although in fact Bradley & Grahame (1950) are at variance with this view. In the specimens examined in this work no glands comparable in any way to mammalian Brunner's glands were found. It is possible, however, that the absence of these glands and the absence of a structure comparable to the mammalian pylorus is compensated from a functional point of view by the abundance

of mucus-secreting cells in the surface epithelium and superficial parts of the glands in the transitional zone interposed between the gizzard and duodenum.

Whether the large granular cells and the small agranular cells in the glands in the transitional zone represent different functional states of the same cell type has not been determined, nor has it been possible to characterize them beyond noting their reaction in preparations stained by the Gomori technique. No zymogenic cells have been demonstrated in this zone.

The goblet cells of the avian intestine have been studied by Cloetta (1893) and Ackert, Edgar & Frink (1939). Zietschmann (1911) described cells with a cuticular border, and Moog (1950) demonstrated alkaline phosphatase in the striated cuticular border in the epithelial cells of the chick duodenum. The striated border of the epithelial cells is comparatively prominent in the chick and the goblet cells, which are scattered singly amongst the cells with striated borders, appear to be most numerous in the colon and least numerous in the caecum. The latter feature may be related to the fluid nature of the contents of these tubes (Browne, 1922). In the blind end of the glands at all intestinal levels all the cells whose cytoplasm reaches the free surface are mucus-secreting.

Looper & Looper (1929) and Calhoun (1954) found numerous eosinophils in the caecum of the chick, but this apparently is not a constant feature. Denke (1954) noted the absence of eosinophils from the intestinal mucosa of the turkey. The statement by Bradley & Grahame (1950) that Paneth cells are present at all levels in the chick has not been confirmed, and it is suggested that the argentaffin cells may have been mistaken for Paneth cells. These are unusually eosinophilic in the chick and readily distinguished even in haematoxylin and eosin preparations. According to Calhoun (1954), Cloetta (1893) doubted the presence of Paneth cells in the intestine of the chick, whilst Greschick (1922) and Clara (1926, 1927) agree with the view expressed by Bradley & Grahame (1950).

Argentaffin cells

The distribution of argentaffin cells in the chick intestine is generally similar to that characteristic of the intestine of some mammals, e.g. mouse (Schofield, 1952), dog, cat, rat (personal observation) in which a high concentration in the upper duodenum is followed by a fall which in turn is succeeded by a rather smaller rise in the colon. Denke (1954) recorded a similar distribution in turkey poult, and although Simard & van Campenhout (1932) reported that 2 days after hatching argentaffin cells were more numerous in the large intestine than in the duodenum it is possible that this apparent contradiction is due to the fact that the zone in the duodenum in which argentaffin cells are particularly abundant is very narrow and may have been overlooked by these authors. In man, however, the distribution of argentaffin cells appears to differ slightly from that noted in the chick, the fall in concentration in the small intestine not being reversed in the colon (Schofield, 1953).

The occurrence of numerous argentaffin cells in the surface epithelium and in the glands towards their open ends, however, is somewhat unusual, these cells in the mammal generally being located mainly in the proximal one-third of the glands. In older birds the distribution may be similar to that noted in the mammal, but the number of birds examined is not sufficient to justify an assumption that dis-

tribution in the chick varies with age, although this is clearly a possibility. It is interesting, however, that one of Simard & van Campenhout's (1932) photographs showing a section of large intestine from an 18-day embryo demonstrates a distribution similar to that noted above in younger birds.

'Migrating' cells apparently moving towards the gland lumen are a feature of other species and do not appear to be particularly frequent in the chick. Simard & van Campenhout (1932), however, observed argentaffin cells in the lamina propria of the embryonic chick intestine and concluded that they had migrated from the epithelium. They were able to demonstrate such migration only at approximately 269 hr. of development, however, and although they are not explicit on this point argentaffin cells do not appear to be demonstrable as such in the lamina propria after this time. Certainly they are not present in birds varying in age from 6 weeks to 18 months, and must therefore either migrate beyond the gut wall or lose their argentaffin granules during development. The intraepithelial location of argentaffin cells in man has been recorded by Schofield (1953).

Schofield (1952, 1953) described morphological differences between the argentaffin cells of the small and large intestines in mouse and man, and advanced the hypothesis that these differences are associated with functional differences. In this respect the chick differs sharply from man and mouse, there being no morphological distinction between argentaffin cells at different intestinal levels in this species, a feature which may be related to the close similarity in structure between these two parts of the intestine.

In his study of argentaffin cells in man Schofield found that the number of reactive cells remained the same whether or not a reducing agent was employed (i.e. the cells are argentaffin rather than argentophile), but that the number of granules in the individual cell was greater when the silver bath was followed by reduction and that only in such circumstances were granules located in the luminal cytoplasm. In the chick also the number of reactive cells is not affected by the use of a reducing agent, but in this species reactive granules are found in the luminal cytoplasm even in methenamine silver preparations. The number of argentaffin granules in this location varies from a very few to such numbers that they completely fill the cell and they may be so closely packed that individual granules cannot be distinguished. It seems unlikely therefore that in the chick the granules pass through a preliminary argentophile phase as suggested by Schofield in man. In this species, therefore, as in man, guinea-pig and mouse (Schofield, 1953, 1950, 1952), there does appear to be a gradual accumulation of granules suggesting the possibility that a secretory cycle is involved, and this is supported to some extent by the fact that occasional cells are found containing only a few granules, distributed throughout the distal cytoplasm from the nucleus to the free border and suggestive of a cell in which a discharge of granules is taking place. There is, however, no direct evidence other than this of exocrine secretion by the argentaffin cells.

Schofield (1950) has described a transformation of the supranuclear granules of the argentaffin cells of the small intestine of the guinea-pig into an argentaffin network containing a mucicarmine-positive substance in its meshes, and he has also described a series of transformations between this cell and the typical goblet cell. This author also found evidence of the transformation of argentaffin cells into

goblet cells in the small intestine of man and mouse (Schofield, 1953, 1952). In the chick the argentaffin cells do not contain mucicarmine-positive material at any stage in development, nor is there any evidence in this species of the reciprocity between the number of argentaffin cells and goblet cells noted by Schofield in man, mouse and guinea-pig. Again in the chick argentaffin cells occasionally occur in small groups of 2-3 cells and are not invariably isolated from one another as in man, mouse and guinea-pig. It is reasonable to suppose, therefore, that in the chick the argentaffin cells are not the source of goblet cells.

Schofield (1953) also described vacuolation of the proximal cytoplasm in argentaffin cells in man and has suggested this may be due to post-mortem change consequent on delay in penetration of the fixative. In the chick intestine, cells showing a slight degree of vacuolation of the proximal cytoplasm were noted occasionally, but were very rare indeed and this may be related to the fact that the maximum delay between death of the bird and fixation was less than 30 min. in every case and generally only a few minutes. Moreover, fixation was carried out in a processor with constant agitation of fixing solution.

Argentaffin cells have rarely been found in the stomach of the chick, but Simard & van Campenhout (1932) did find some in the proventriculus in one case. Dawson & Moyer (1948), on the other hand, found these cells to be constantly absent from the gizzard and proventriculus. It seems unlikely, therefore, that the occasional argentaffin cells found in this situation are functionally significant.

SUMMARY

1. The secreting cells of the submucosal glands of the proventriculus present features which suggest that they are concerned with HCl production.
2. The secretion of the glands of the gizzard gives histochemical reactions which suggest that it is a form of hard keratin. It presents certain features, however, which distinguish it from the other keratinous structures of the body.
3. A transitional zone with distinctive features situated between the gizzard and duodenum has been described. It is suggested that this bears some functional resemblance to the mammalian pylorus.
4. The distribution and morphology of the argentaffin cells in the chick have been described. These cells appear to be morphologically similar in small and large intestine, a feature which may be related to the general similarity between these two parts of the intestine in the chick. There does not appear to be any connexion between the argentaffin cells and the intestinal goblet cells in the chick.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Submucosal glands of proventriculus. Gomori's trichrome. $\times 120$.

Fig. 2. Proventriculus. Secreting cells of a submucosal gland. Gomori's trichrome. $\times 1100$.

Fig. 3. Gizzard. PAS. $\times 120$.

Fig. 4. Gizzard to show reaction of cells lining the gland tubule towards its open end.

PLATE 2

Fig. 5. Gizzard. Azure A–eosin B after oxidation by peracetic acid. $\times 120$.

Fig. 6. Gizzard–duodenal junction. Southgate's mucicarmine. $\times 120$.

Fig. 7. Gizzard–duodenal junction. Argentaffin cells are particularly abundant in the duodenum which lies in the upper half of the photograph. Gomori's methenamine-silver. $\times 120$.

Fig. 8. Duodenum. Southgate's mucicarmine. $\times 120$.

Fig. 9. Small intestine. Numerous argentaffin cells can be distinguished in the surface epithelium.
Gomori's methenamine-silver. $\times 100$.

PLATE 3

Fig. 10. Small intestine. To show 'migrating' argentaffin cell. Gomori's methenamine-silver.
 $\times 1000$.

Fig. 11. Small intestine. Ferric ferricyanide reduction. $\times 1600$.

Fig. 12. Large intestine. Gomori's methenamine-silver. $\times 1600$.

Fig. 13. Large intestine. Gomori's methenamine-silver. $\times 1600$.

Fig. 14. Caecum. Gomori's methenamine-silver. $\times 1600$.



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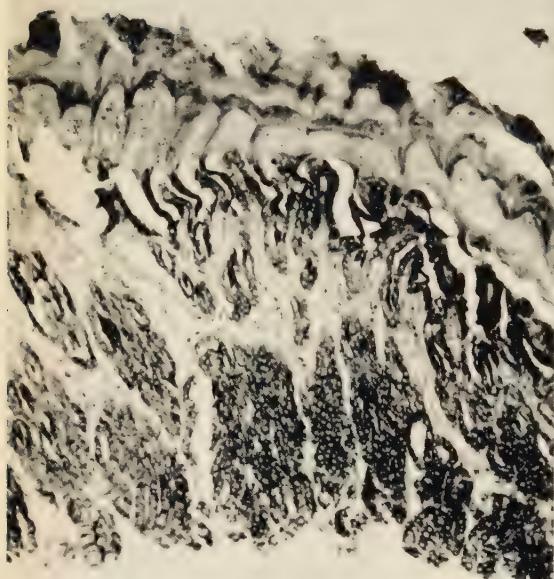
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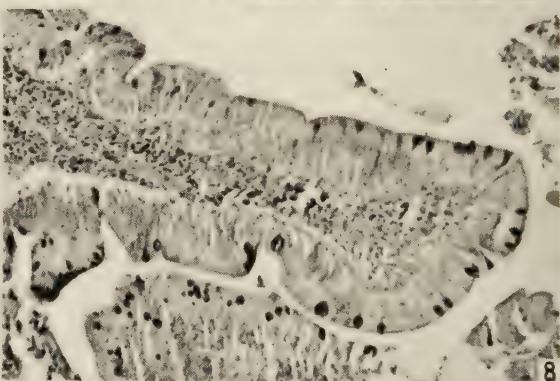
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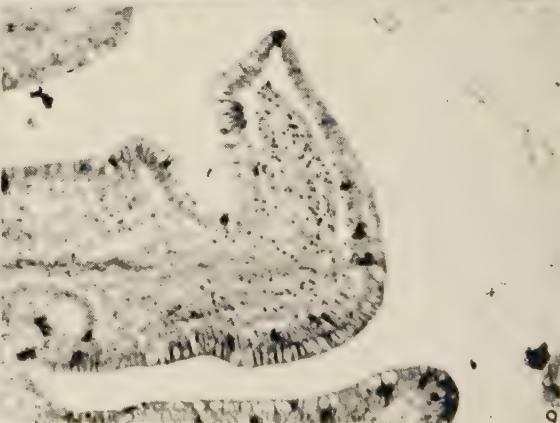
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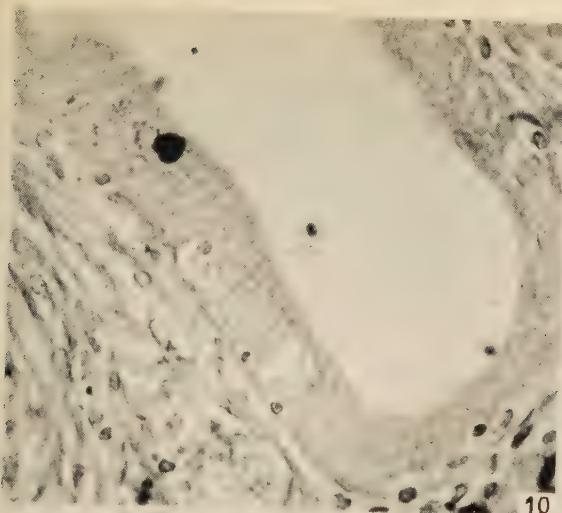
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AN AUTORADIOGRAPHIC AND HISTOCHEMICAL
STUDY OF LONG-TERM CARTILAGE
GRAFTS IN THE RABBIT

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Two conflicting views have been expressed as to the fate of the cells in any graft of fresh, viable cartilage. It has been stated that, on the one hand, the cells die shortly after grafting, an opinion based mainly on clinical experience (Maumenee, 1953; Longmire, Cannon & Weber, 1953; Billingham, 1954, 1955); on the other hand, that they remain alive for considerable periods. This latter view is based partly on the normal histological appearance presented by autografts (Peer, 1946) and homografts (Bacsich & Wyburn, 1947; Peer, 1954) and partly on evidence from more functional experiments. Dupertuis showed that cartilage autografts and homografts in rabbits (1941) and autografts in man (1950) will exhibit growth after transplantation if taken from a young donor. In rabbits, cartilage autografts and homografts have been shown to possess a measurable carbohydrate metabolism for up to 150 days after grafting (Laskin, Sarnat & Bain, 1952), and the cells of both types of graft contain normal lipid and glycogen deposits after 2 years (Craigmyle, 1954, 1955).

Radioactive sulphur administered as sulphate is rapidly incorporated into cartilage *in vivo* (Dziewiatkowski, Benesch & Benesch, 1949; Odeblad & Boström, 1952; Davies & Young, 1954; Pelc & Glücksmann, 1955). It appears in the first instance in the cells (Pelc & Glücksmann) and occurs finally as the ester sulphate of the chondroitin sulphuric acid of the matrix. Purified chondroitin sulphate alone will not incorporate $^{35}\text{SO}_4$ *in vitro* (Boström, 1952). It has been clearly shown that utilization of $^{35}\text{SO}_4$ by chick (Layton, Frankel & Scapa, 1950), bovine (Boström & Mansson, 1952, 1953) and human (Curran & Gibson, 1956) cartilage is dependent on the presence of living cells and does not occur in cartilage killed by heat or chemical poisons. Using this test of viability, Wyburn & Bacsich (1955) and Gibson, Curran & Davis (1957) demonstrated living cells in cartilage homografts after 3 weeks in guinea-pigs and 15 months in man respectively; and Davis & Gibson (1957) in human cartilage autografts after 37 years. No account has been published as yet of the uptake of $^{35}\text{SO}_4$ by cartilage heterografts.

The present study concerns the incorporation of $^{35}\text{SO}_4$ by long-term autogenous, homogenous and heterogenous cartilage grafts in the rabbit, and at the same time compares some of the histochemical reactions of the matrix of the grafts with those of normal cartilage of the host animal.

MATERIAL AND METHODS

Seven rabbits were used. Each had received approximately 2 years previously, under Nembutal anaesthesia, multiple block grafts of fresh costal cartilage as shown in Table 1.

Twenty-four hours before attempted recovery of the grafts, each animal was given a subcutaneous injection of 1 ml. of $\text{Na}_2^{35}\text{SO}_4$ containing 5 mc. of ^{35}S . All grafts recovered were fixed in 10% neutral formaldehyde, and embedded in paraffin. Sections were cut at $7\ \mu$, and autoradiographs made using the stripping film method with an exposure of 90 days. This was performed on an autograft sample from each of animals L 293 and R 338, and also on one or more graft samples from each animal in which foreign cartilage had persisted. As a control, a piece of costal cartilage from a young adult rabbit was incubated at 37°C . for 24 hr. in 2 ml. Ringer solution containing 5 mc. of ^{35}S . It was then fixed in 10% formalin and autoradiographs made. Other sections of the grafts, and of a piece of costal cartilage from L 293 fixed in 10% formalin were stained with (1) haematoxylin and chromotrop; (2) 0.5% aqueous alcian blue; (3) 0.01% aqueous toluidin blue; and (4) the periodic acid Schiff reaction.

Table 1

Animal no.	Type(s) of graft	Site of grafting	Exact duration of experiment (days)
L 293	Auto- and Homografts	Intramuscular	659
R 338	Auto- and Homografts	Intramuscular	671
L 290	Homografts	Subcutaneous	667
L 291	Homografts	Subcutaneous	757
L 269	Heterografts*	Intramuscular	679
R 351	Heterografts*	Intramuscular	684
R 370	Heterografts*	Subcutaneous	731

* Obtained from freshly killed guinea-pigs.

RESULTS

Each autograft and homograft was recovered and appeared to have undergone no macroscopic change. Of all the heterografts implanted, however, only a small fragment of one was found to have persisted for 679 days in animal L 269: in the other two rabbits all the guinea-pig cartilage grafts had been completely absorbed. All autografts and homografts, and the small fragment of the heterograft recovered, appeared to consist histologically of normal healthy cartilage. Degeneration of the central regions was seen in many of the grafts, however.

The control piece of cartilage incubated in a medium containing $^{35}\text{SO}_4$ gave a strongly positive autoradiograph manifested by blackening of the film overlying the cartilage (Pl. 1, fig. 1). The greatest concentration occurred over the mature chondrocytes towards the centre of the tissue, whilst the superichondral chondroblasts and the immature chondrocytes showed little activity. (The dark appearance of these cells in Pl. 1, fig. 1, is due to some refraction and not to silver granules.) The matrix generally gave a very faintly positive result, and it would appear that 24 hr. after administration the bulk of the isotope is present within the cartilage cells: this is in agreement with the findings of Pele & Glücksmann (1955). Every graft studied autoradiographically gave a positive result (Pl. 1, figs. 2-4), but the fixation of the

isotope by the graft cartilage was much less intense than by the control cartilage as would be expected since the concentration of the isotope was much greater in the incubating medium than in the rabbit tissue fluid. The strongest activity, and in the case of some grafts, the sole activity, was found in the young adult cartilage cells in a zone some distance from the perichondrium, whilst the subperichondral chondroblasts and, in contrast to the control, the oldest chondrocytes towards the centre of the graft showed little or no activity (Pl. 1, fig. 3). The matrix of all the grafts showed negligible content of the isotope.

With regard to the outcome of the histochemical tests employed for the demonstration of acid mucopolysaccharides, normal cartilage was found to possess a particularly strong localized concentration of acid mucopolysaccharide in the cell capsules—the matrix in the immediate vicinity of the cells—since staining with alcian blue, metachromasia with toluidin blue and the PAS reaction are all more intense in this region than in the matrix elsewhere (Pl. 1, figs. 5, 7 and 9). The grafted cartilage gave fundamentally similar staining reactions, but there was a general tendency for the matrix of the grafts to show a somewhat weaker staining response to alcian blue, and reduced metachromasia with toluidin blue (Pl. 1, figs. 6, 8). This was especially true of the general matrix, but less marked in the cartilage capsules. With the PAS reaction, on the other hand, the matrix of the grafts either showed no difference from the control or stained even more intensely (Pl. 1, figs. 9, 10). It has, however, to be borne in mind that the specificity of the PAS reaction for acid mucopolysaccharides is generally accepted to be considerably less than that of the two other histochemical techniques employed.

DISCUSSION

This study confirms previous findings by the author (Craigmyle, 1955) that cartilage autografts and homografts persist unchanged for up to 2 years in rabbits, and establishes further that the cells in both types of graft have remained viable as evidenced by their capacity to utilize $^{35}\text{SO}_4$. This holds good also for the heterograft fragment. In other words, as long as histologically normal and fully stainable cartilage is present, the chondrocytes therein, whether autogenous, homogenous or heterogenous, are viable. Cartilage cells are almost unique, therefore, in their ability to remain alive for at least 2 years in a foreign host. It is, of course, not without significance that the heterografts were found to have been almost totally absorbed whilst the homografts remained unchanged. A possible, and perhaps the most likely explanation, is that this is due to the cartilage heterografts being effectively antigenic. This would tie up well with previous observations made on the impact of cartilage homo- and heterografts on the regional lymph nodes (Craigmyle, 1958), when the heterografts produced striking increases in weight and cytological detail of the regional nodes whilst homografts were practically ineffective: at best the homografts showed a very mild degree of antigenicity. Similarly, cartilage homografts appear to produce only slight 'sensitization' of the host to a subsequent skin homograft taken from the same donor (Craigmyle, 1957).

The histochemical staining reactions of the matrix of all grafts recovered showed them to possess a near normal or somewhat reduced amount of acid mucopoly-

saccharide as compared with that of ungrafted costal cartilage taken from one of the host animals. In connexion with these findings it is pertinent to recall Hass's (1943) work on ageing cartilage in which he states: 'The number of cells in the matrix diminishes and those which remain display morphologic changes which are ordinarily accepted as evidence of diminished activity, if not of decreased viability. There is little doubt that one function of the cells is synthesis of polysaccharide or its precursors and it is reasonable to assume that the limitation of this function in aged cartilage is responsible for the low polysaccharide values. In other words, the preservation of the matrix in a youthful state is contingent on the continuous replacement of polysaccharide, and if this does not occur, the quantity of polysaccharide decreases.' In the light of this, the present study may indicate that the graft cartilage has aged more rapidly than has ungrafted cartilage in the host animal. The central degeneration seen in many of the grafts is not necessarily evidence of accelerated ageing, however, as it is a common pre-ossification finding in ungrafted costal cartilage.

SUMMARY AND CONCLUSIONS

An autoradiographic and histochemical study has been made of cartilage autografts, homografts and heterografts implanted in rabbits for periods ranging between 659 and 757 days. The autogenous and homogenous grafts were found unchanged, but the heterogenous cartilage had been almost completely absorbed. A small fragment of only one such graft was recovered.

Autoradiographic studies using $^{35}\text{SO}_4$ indicate that many of the cells in all three types of graft are alive as evidenced by their ability to metabolize sulphur administered as sulphate. The histochemical reactions for acid mucopolysaccharides of the matrix of the grafts were either normal or differed only little quantitatively from those of the matrix of ungrafted cartilage of the host animal. It is considered that the grafts recovered consisted of viable cartilage.

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EXPLANATION OF PLATE

Fig. 1. Young adult rabbit. Control cartilage after incubation in a medium containing ^{35}S . Autoradiograph. High concentrations of ^{35}S in the mature cartilage cells in the centre of the graft (below). The dark appearance of the immature chondrocytes (top) is an artefact due to refraction. $\times 105$.

Fig. 2. Animal L291. Homograft after 757 days and 24 hr. after administration of ^{35}S . Autoradiograph. Aggregations of granules can be seen over nearly every mature cartilage cell whilst the perichondrium is negative. $\times 27$.

Fig. 3. Animal R 338. Homograft after 671 days and 24 hr. after administration of ^{35}S . Autoradiograph. The greatest uptake of ^{35}S is by the young adult chondrocytes in a zone below the perichondrium, whilst the cells of the perichondrium (top) show no uptake, and the oldest chondrocytes (below) show little. $\times 240$.

Fig. 4. Animal L 269. Heterograft after 679 days and 24 hr. after administration of ^{35}S . Autoradiograph. Uptake of ^{35}S by the graft cells. $\times 470$.

Fig. 5. Control costal cartilage from animal L 293. Toluidin blue. Metachromasia of the matrix is of patchy nature and is greatest in the cell capsules. $\times 85$.

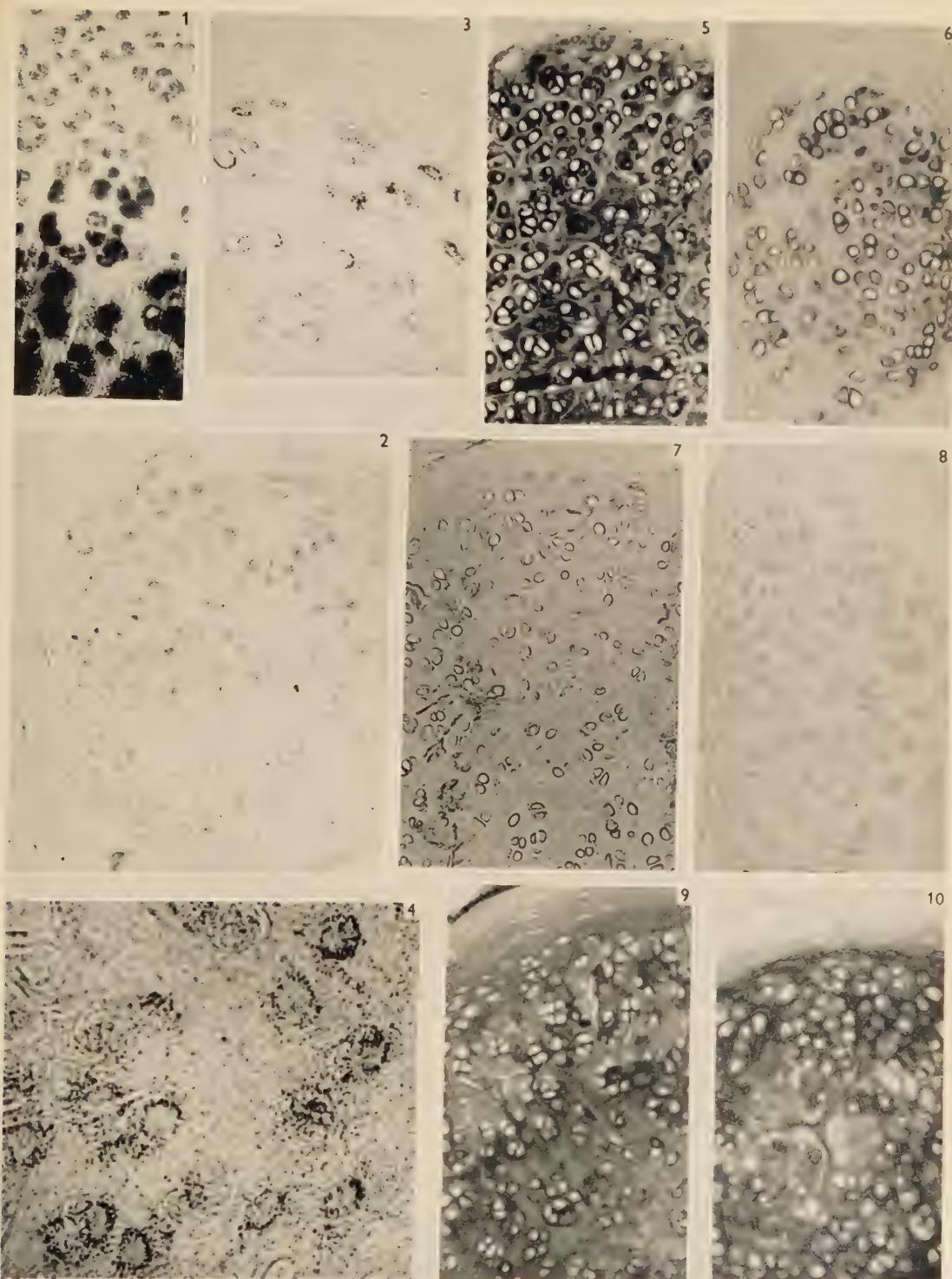
Fig. 6. Animal L 291. Homograft after 757 days. Toluidin blue. The degree of metachromasia is less than in fig. 5. $\times 95$.

Fig. 7. Control costal cartilage from animal L 293. Alcian blue. Staining intense in the cartilage capsules and less intense in the matrix elsewhere. $\times 85$.

Fig. 8. Animal L 291. Homograft after 757 days. Alcian blue. Reduction in intensity of staining as compared with fig. 7. $\times 92$.

Fig. 9. Control costal cartilage from animal L 293. PAS reaction. The cell capsules are strongly positive, the general matrix less so. $\times 85$.

Fig. 10. Animal L 291. Homograft after 757 days. PAS reaction. There is no loss of staining intensity by comparison with fig. 9, in fact, if anything, this graft is stained more intensely than the control. $\times 105$.



THE ANNULAR LIGAMENT OF THE SUPERIOR RADIO-ULNAR JOINT

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INTRODUCTION

There is a fair degree of uniformity in the descriptions in textbooks of anatomy of the form and arrangement of the annular (coronary) ligament of the superior radio-ulnar joint. The usual conception of this ligament is that it consists of a fibrous band, forming about four-fifths of a circle, attached to the anterior and posterior margins of the radial notch of the ulna: above, the ligament is continuous with the capsule of the elbow joint, and below, where its diameter narrows, it grasps the neck of the radius (thereby preventing downward displacement of the radial head), and is attached to it by a thin membranous layer. Allowing for slight variations in individual accounts, this is the version given by Bryce (1915), Wood Jones (1949), Grant (1948), Walmsley (1951), Terry & Trotter (1953), Johnston & Whillis (1954) and Last (1954).

It is generally accepted that the lateral ligament of the elbow joint descends from the lateral epicondyle of the humerus, spreads out fanwise over the external surface of the annular ligament and blends with it, but some accounts make the additional observation that the fibres of the anterior and posterior parts of the lateral ligament are continued across the annular ligament to become attached to the ulna, in front of and behind the radial notch, thereby linking humerus and ulna (e.g. Bryce, 1915; Vallois, 1926; Wood Jones, 1949; Walmsley, 1951). Vallois (1926) stressed the importance of the contribution made by these fibres to the annular ligament, and in fact was of the opinion that they form the major part of it, the deeper annular fibres being less numerous and less important; separation of the latter into a distinct ligament he considered to be somewhat artificial.

The present work has shown that the superior radio-ulnar joint possesses its own capsule, continuous above with that of the elbow joint, and that it is reinforced externally by a number of ligaments which blend with it and with one another, and thus contribute to the formation of the annular ligament. The ligaments are stronger and have a more extensive attachment to the ulna posteriorly than anteriorly.

There are certain observations recorded in some older works which are in accord with the present findings, and these will be referred to in the discussion.

MATERIALS AND METHODS

Dissections were made of the superior radio-ulnar joint of thirty specimens, obtained from the dissecting room after the main dissection of the upper limb had been completed. A study was also made of the proximal ends of twenty dried radii and forty dried ulnae, to determine the relationship of the various parts of the annular ligament to these bones.

RESULTS

It was found that the ligaments of the superior radio-ulnar joint lie in three strata. The innermost stratum is provided by the capsule of the joint, and is recognizable as a separate entity posteriorly. The intermediate stratum forms an annular band, broader posteriorly than anteriorly, and is the structure to which the term 'annular ligament' has been applied in most anatomical works. The outermost stratum is derived from the lateral ligament of the elbow joint, which spreads out fanwise over the outer surface of the intermediate stratum, and commonly both its anterior and posterior fibres are continued downwards to gain attachment to the ulna.

The three ligamentous strata are distinguishable posteriorly, but anteriorly the two deeper ones are blended, so that the arrangement appears more complex posteriorly. Furthermore, the ligaments have a larger area of attachment to the ulna posteriorly than anteriorly; in some cases, the length of the posterior attachment is as much as 2·5 cm. It is advisable, therefore, to consider separately the anterior and posterior arrangement of the ligaments which, by blending together, produce the annular ligament.

(a) The posterior arrangement

From the thicker, posterior part of the lateral ligament of the elbow joint, a bundle or band of fibres continues downwards and backwards across the intermediate stratum of the annular ligament and gains attachment to the lateral side of the ulna (*L.* in Fig. 1 (5, 6); Fig. 2 (7, 8, 10, 11)). Particular attention was paid to the ulnar attachment of this bundle, and in reference to it a study was made of forty dried ulnae. It was found that the fibres are attached in most instances to a special tubercle, situated on or a little in front of the supinator crest, about 1 cm. below the radial notch. The tubercle varies in size from a small conical elevation to a well-marked shelf (*T.* in Fig. 1 (1, 2)). An obvious tubercle is not present in all ulnae, however, and the upper part of the supinator crest may be salient instead (*S.* in Fig. 1 (3)). These differences in ulnar marking reflect the differences encountered in the degree of development of the attached fibrous bundle or band.

Although in most cases the fibres attached to this part of the ulna are in direct continuity with the lateral ligament of the elbow joint, in some specimens a proportion of the fibres, when traced upwards from their ulnar attachment, blend with the lower part of the annular ligament (*L.* in Fig. 1 (5); Fig. 2 (7, 8, 10, 11)): in such cases, the fibrous bundle really constitutes a separate ligament, arising from the ulna and blending partly with the lateral ligament of the elbow joint and partly with the lower part of the annular ligament. In two specimens some of these fibres actually passed deep to the intermediate stratum or annular band (Fig. 1 (6); Fig. 2 (11)).

The intermediate stratum is the strong band of fibres arranged in annular fashion around the head of the radius and, as stated above, it is the structure to which the term 'annular ligament' is usually applied.

It was found that in many cases this stratum gains attachment to the ulna posteriorly, not to the posterior margin of the radial notch as commonly asserted, but to a rough ridge situated 2-3 mm. behind the notch, and in line with the supinator crest (*R.* in Fig. 1 (1); compare with Fig. 2 (9)). In only about one-half

of the ulnae examined was the articular surface of the radial notch carried on to the anterior face of this ridge (Fig. 1, (2, 3)).

Although this band has been invariably described as a single structure, it was found in the present study that there is considerable variation in the form and extent of its attachment posteriorly. In only one-half of the specimens was it attached as a single band (*AN.* in Fig. 1 (5); Fig. 2 (11)), and even in about one-half of these, the band was joined from above by a narrow ligament which arose from the lateral side of the ulna opposite the middle of the trochlear notch (*U.* in Fig. 1 (6)); in other cases the band was subdivided posteriorly, prior to its attachment, into two, three

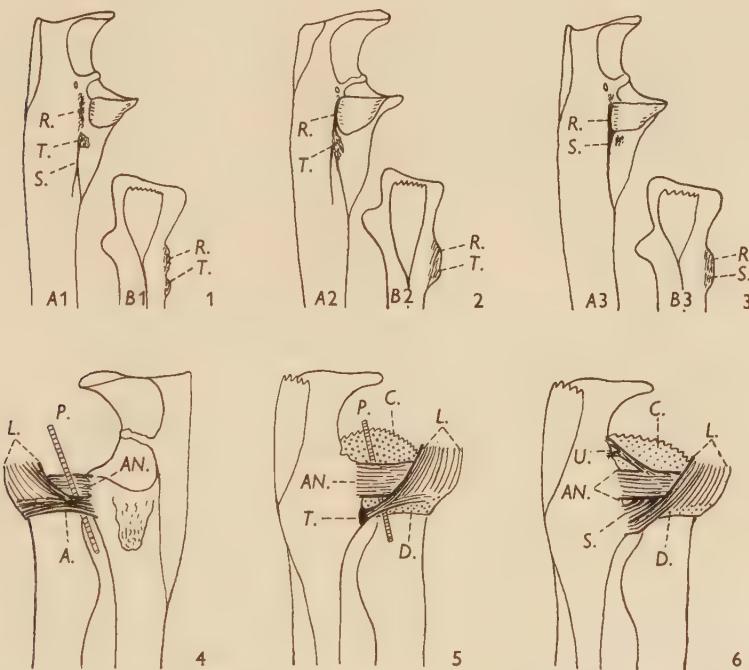


Fig. 1

- (1) Lateral (*A*1) and posterior (*B*1) aspects of ulna. Note tubercle just in front of supinator crest, and rough ridge behind radial notch.
- (2) A large tubercle is present on the upper part of the supinator crest, and it is continuous with the rough ridge or shelf, bounding the radial notch posteriorly.
- (3) In this ulna, no special tubercle is present, but the upper part of the supinator crest is salient and continues into the ridge bounding the radial notch posteriorly. The 'deep band' gains attachment to the small roughened area shown in the floor of the bicipital fossa (see (9), Fig. 2).
- (4) The anterior ligament blends mainly with the lower part of the annular band, but some fibres are continuous with the anterior part of the lateral ligament of the elbow joint.
- (5) Posterior aspect of the annular ligament. The annular band is attached to the ulna as a single structure. The lateral ligament of the elbow joint blends with its outer surface, and its posterior fibres gain attachment to the special tubercle. The capsule of the elbow joint passes deep to the annular band and, as the 'deep band', forms the lower part of the annular ligament.
- (6) Annular band is joined from above by a slender slip. Some fibres from the supinator crest pass deep to the annular band; the remainder cross superficially and join the lateral ligament of the elbow joint.

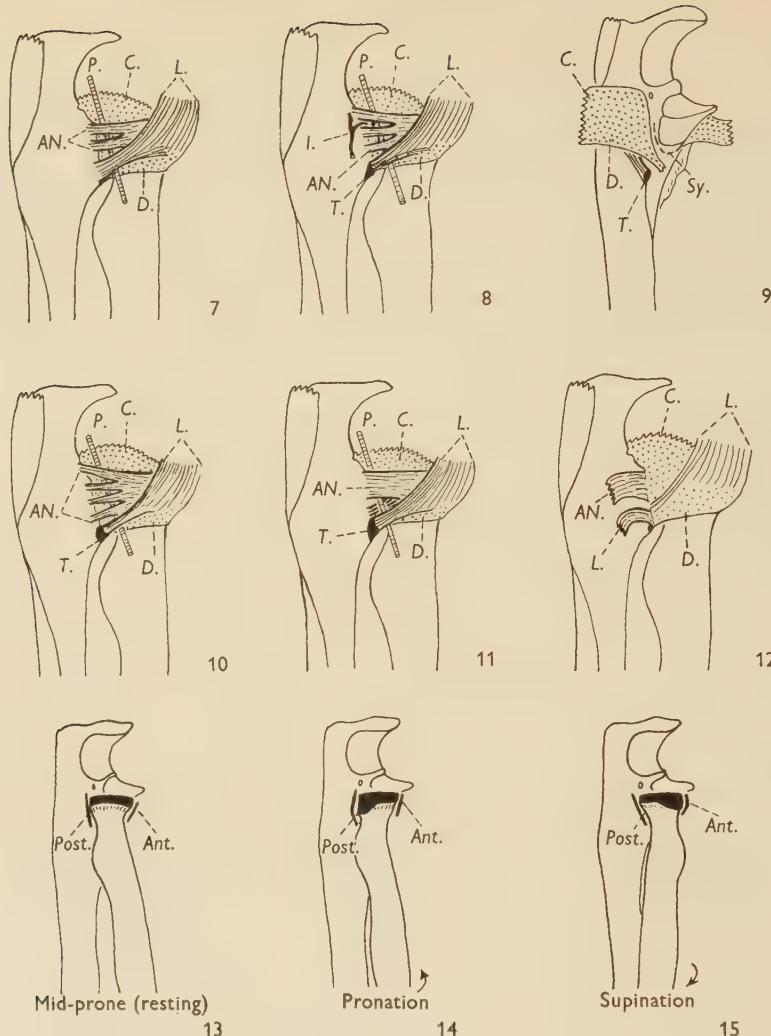


Fig. 2

- (7) The annular band is subdivided into two equal slips posteriorly.
- (8) The annular band is subdivided into three equal slips posteriorly.
- (9) Same specimen as in (8); annular ligament divided and capsule of elbow joint trimmed parallel to upper border of annular band. The annular ligament is wider posteriorly than anteriorly. The 'deep band' is attached to the floor of the bicipital fossa, and is continuous above with the capsule of the elbow joint. Anteriorly, the annular ligament is attached to the anterior sloping margin of the radial notch. Note reflexion of the synovial membrane.
- (10) The annular band is subdivided into four equal slips posteriorly.
- (11) The annular band is attached posteriorly as a single structure. Though most fibres attached to the special tubercle pass upwards superficial to the annular band, some pass deep to it.
- (12) Same specimen as in (11). The annular band and the posterior fibres of the lateral ligament of the elbow joint have been divided and reflected. The downward continuation of the capsule of the elbow joint is seen, and closely applied to it are the fibres which passed from the special tubercle, deep to the annular band.
- (13) Radius in the mid-prone (resting) position: neither the anterior nor the posterior part of the annular ligament is under tension.
- (14) Radius in prone position: posterior part of the annular ligament is under tension.
- (15) Radius in supine position: anterior part of the annular ligament is under tension.

or even four parts, more or less equal in size (*AN.* in Fig. 2 (7, 8, 10)). An articular branch or branches of the posterior interosseous recurrent artery passed to the superior radio-ulnar joint between these divisions of the annular band (*I.* in Fig. 2 (8)).

In nearly every case, a probe could be passed between the annular band near its posterior attachment and the underlying joint capsule or 'deep band' (*P.* in Fig. 1 (5); Fig. 2 (7, 8, 10, 11)), but a little further laterally these two strata blend together. If the annular band is divided posteriorly and reflected, the 'deep band' is exposed (*D.* in Fig. 2 (12)).

The 'deep band', which forms the innermost stratum of the annular ligament, is attached to the ulna posteriorly, just in front of the annular band. The interval between these two strata is rather greater inferiorly because here the attachment of the 'deep band' moves forwards on to the floor of the bicipital fossa, a point which can be particularly well seen after the joint has been opened and the radius removed (*D.* in Fig. 2 (9)). The lower part of the 'deep band' is thus brought into apposition with the neck of the radius, and it forms the major portion of the lower part of the annular ligament, i.e. the part which grasps the neck of the radius (*D.* in Fig. 1 (5, 6); Fig. 2 (7-12)). The upper part of the 'deep band', which surrounds the head of the radius, is thinner than this lower part, but it is reinforced by the overlying annular band.

(b) *The anterior arrangement*

As can be seen in the opened superior radio-ulnar joint (Fig. 2 (9)), the anterior part of the annular ligament is narrower than the posterior part. It is attached, as a single band, to the anterior margin of the radial notch, and since this margin slopes downwards and backwards, the lower part of the ligament is brought into apposition with the neck of the radius.

The annular ligament appears to be of simpler structure anteriorly than posteriorly because in the former place the 'deep band' is blended with the overlying annular band, and furthermore the annular band does not subdivide here prior to its attachment.

The external stratum is represented anteriorly by a small ligament which arises from the coronoid process, 2-3 mm. anterior to the lower part of the annular band, and passes horizontally outwards to blend with the outer surface of the lower part of the annular band. This anterior ligament was present in nearly every specimen examined, but varied in its degree of development. In most cases it was a thin flat band about 3 mm. in width, but in some specimens it was quite substantial, and then some of its fibres were in direct continuity with the anterior part of the lateral ligament of the elbow joint (*A.* in Fig. 1 (4)). A probe can be inserted between this anterior ligament and the annular band.

In its lowermost part, the annular ligament becomes tenuous and provides but a thin fibrous covering for the underlying synovial membrane, where this is reflected on to the neck of the radius. Although this thin part of the ligament does gain attachment to the neck of the radius, it is very easily detached from the bone. The thinness of the ligament near its attachment allows it to become twisted during the rotatory movements of the radius, and the tension set up in the ligament in this way is one (though a minor) factor concerned in limiting pronation and supination.

No evidence was found for the existence of a quadrate ligament closing the inferior aspect of the superior radio-ulnar joint; the synovial recess between the radius and ulna was found to be covered externally by nothing more than a thin fibrous layer, which can scarcely be referred to as a ligament.

THE EFFECTS OF PRONATION AND SUPINATION ON THE ANNULAR LIGAMENT

It was observed that when the forearm bones are in the mid-prone position, neither the anterior nor the posterior parts of the annular ligament are under tension. In full pronation, however, the posterior part of the ligament becomes tense, especially its lower part (the 'deep band'), whilst the anterior part slackens. In full supination, the reverse occurs, and the anterior part of the ligament becomes tense whilst the posterior part slackens.

These effects were found to depend upon the shape of the head and neck of the radius. In the first place, it is known that the head of the radius is not quite circular. According to Vallois (1926), the antero-posterior diameter is 1-1.5 mm. greater than the transverse, and this was confirmed in the present investigation. When the forearm bones are in the mid-prone position, the narrower transverse diameter of the radial head is brought to the antero-posterior position, so that tension on both anterior and posterior parts of the annular ligament is reduced. Furthermore, in this position, the neck of the radius presents to all parts of the annular ligament a surface which slopes downwards and inwards, and the prominent articular portion of the rim of the radial head faces the radial notch of the ulna (Fig. 2 (13)).

In pronation, the articular prominence of the radial head is partly brought out of the radial notch and becomes pressed against the posterior part of the annular ligament. In addition, just below the prominence the neck of the radius is scarcely sloped at all, so that also is pressed against the posterior part of the annular ligament, especially the 'deep band' (Fig. 2 (14)). The opposite side of the radial head and neck, now in contact with the anterior part of the annular ligament, is quite differently shaped; the rim of the head is narrow, and the neck below it slopes markedly, consequently the anterior part of the ligament is not put under tension. In supination, the reverse conditions are obtained, and the anterior part of the ligament is put under tension whilst the posterior part slackens (Fig. 2 (15)).

DISCUSSION

This study has shown that the superior radio-ulnar joint has the same fundamental structure as other synovial joints, in so far as it possesses a capsule, which is lined by synovial membrane and reinforced externally by accessory ligaments. Since the joint is continuous with the elbow joint, it is not surprising to find that in addition to the continuity of the synovial membrane between the two joints, they share a common capsule, and also an accessory ligament, namely, the lateral ligament of the elbow joint.

Thus, what is described in the majority of anatomical works as a single band surrounding the head and neck of the radius, and named the annular ligament, is in fact a composite structure, formed from the ligaments of the superior radio-ulnar

joint. Whether all the ligaments associated with the joint should be included under the one heading of 'annular ligament', as has been done in the present report, is of course open to question. The joint capsule and the overlying annular band blend together as they are traced forwards from their posterior attachments, and the capsule (the 'deep band') alone forms the lower part of the annular ligament posteriorly. It would seem reasonable therefore to include both these fibrous strata under the same heading. The ligaments of the outermost stratum could perhaps be regarded as anterior and posterior accessory ligaments. These are in the same plane as, and commonly continuous with, the lateral ligament of the elbow joint. However, in some instances, and particularly in the case of the smaller anterior ligament, it was found that as they are traced back from their ulnar attachments, they contribute fibres to the lower part of the annular ligament: sometimes, indeed, no continuity with the lateral ligament is observed, but this is more often the case with the anterior than the posterior ligament.

Although most descriptions of the annular ligament do not give a clear indication of its composite nature, certain of the observations made in the present investigation were recorded in older anatomical works. Reference should be made in particular to the accounts given by Humphry (1858), Morris (1879), and Henle (1871). Both Humphry (1858) and Morris (1879) noted that the annular ligament is attached not only to the anterior and posterior margins of the radial notch of the ulna, but also by bundles of fibres to the rough ridge that descends from the hinder end of the notch, and also to a ridge that descends from the inner side of the coronoid process near the anterior edge of the notch. Although not clearly described in the text, an anterior accessory ligament is illustrated in one of Humphry's diagrams, arising from the coronoid process, but blending with the upper part of the annular ligament and not with the lower part as invariably found in the present study. Neither of these authors (Humphry, 1858; Morris, 1879) described extensions of the lateral ligament of the elbow joint across the annular ligament to the ulna, and, although both described the annular ligament as being connected to the neck of the radius by a thin fibrous layer covering the synovial membrane of the radio-ulnar joint, no reference was made to a proper capsule of the joint. Henle (1871), on the other hand, regarded the annular ligament as a specially thickened part of the capsule of the elbow joint, and stated that the capsule is continued below the level of the ligament as a thin layer which gains attachment to the neck of the radius. He recognized, too, that oblique fibre bundles or accessory ligaments are mixed with the transversely disposed fibres of the annular ligament. Although he did not illustrate the arrangement of these fibres very clearly, he stated that they are attached above and below the radial notch, and take origin from the olecranon and coronoid process. He also described the lateral ligament of the elbow joint as spreading out on the annular ligament, some of its fibres passing forwards and others backwards to gain the ulnar interosseous border; presumably this should be taken to mean the supinator crest, which continues into the interosseous border.

Henle accepted the existence of a quadrate ligament, joining the lower margin of the radial notch to the neck of the radius, and such a ligament is described by many other authors (e.g. Bryce, 1915; Wood Jones, 1949; Walmsley, 1951; Terry &

Trotter, 1953; Johnston & Whillis, 1954). However, the results of the present study substantiate the opinion of Fick (1904) and Vallois (1926) that the thin fibrous covering over the synovial membrane in this position scarcely warrants the status of a ligament. Again, no evidence was found for the existence of fibres continued from the lower part of the annular ligament to surround completely the neck of the radius, as has been described by some authors (e.g. Henle, 1871; Morris, 1879; Terry & Trotter, 1953; Johnston & Whillis, 1954).

The influence of the annular ligament on the movements of pronation and supination was described by Henle (1871) and Morris (1879). Morris stated that the only portion of the ligaments about the elbow joint that could possibly have any effect in controlling the rotation of the radius is the membranous felt-like ligamentous tissue which passes from the lower edge of the annular ligament to the neck of the radius, and the few fibres of the capsule of the elbow joint which are inserted on to the radius; these latter fibres were not observed in the present study. He considered the main factors limiting pronation and supination to be the interosseous membrane, oblique cord (limiting supination), the ligaments of the wrist and inferior radio-ulnar joints, and some of the forearm muscles. Henle (1871) was also of the opinion that the movements of the radius in pronation and supination are limited by the torsion produced in the thin tissue (i.e. joint capsule) extending from the lower part of the annular ligament to the neck of the radius, but he indicated that the tension alternately produced in the anterior and posterior bundles of fibres passing from the ulna to the joint capsule is also a factor. He stated that pronation and supination could scarcely be increased even when the lower ends of the radius and ulna were sawn off and all muscles and connecting tissue between the bones below the elbow joint were removed.

The observations made in the present study have confirmed that the lowermost part of the annular ligament undergoes torsion during the movements of pronation and supination. Furthermore, in agreement with Henle (1871), it was found that the fibres of the annular ligament attached to the ulna behind the radial notch are put under tension in pronation and that those attached in front of the notch are put under tension in supination. It is suggested from the present observations that these changes in tension in the annular ligament depend upon the shape of the head and neck of the radius, but it is not claimed that tension in the annular ligament is the sole factor limiting the movements of pronation and supination.

The reason that the annular ligament is stronger and has a more extensive attachment to the ulna posteriorly is in all probability that the superior radio-ulnar joint is not so well protected by overlying soft parts posteriorly as it is anteriorly.

SUMMARY

1. It has been shown that the annular ligament is a complex structure, formed by the blending of the ligaments of the superior radio-ulnar joint. These ligaments lie in three strata.
2. The innermost stratum is formed by the capsule of the joint, which is continuous superiorly with the capsule of the elbow joint. Inferiorly, the anterior part of the capsule is blended with the overlying intermediate stratum, but the posterior

part (the 'deep band') is separated from it by a slight interval and forms the lower part of the annular ligament.

3. The intermediate stratum is the annular band to which the term 'annular ligament' is usually applied. It is attached anteriorly to the anterior margin of the radial notch of the ulna as a single band, but posteriorly, where it is wider and stronger, it is frequently subdivided into two or more bands which are attached to the posterior margin of the radial notch or to a rough ridge 2–3 mm. behind it.

4. The outermost stratum is derived from the lateral ligament of the elbow joint. Its posterior fibres form a definite bundle which normally crosses the annular band and gains attachment to the supinator crest, frequently to a special tubercle on that crest. The anterior fibres may also cross the annular band and become attached to the coronoid process, though they are frequently replaced by a small ligament which arises from the coronoid process and reinforces the lower part of the annular ligament.

5. No structure warranting the name of a quadrate ligament was observed.

6. The anterior part of the annular ligament is under tension in supination and the posterior part in pronation. These effects depend upon the shape of the head and neck of the radius.

I should like to thank Prof. F. Davies for his helpful criticism in the preparation of the manuscript.

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KEY TO LETTERING OF FIGURES

<i>Ant.</i>	Anterior part of annular ligament
<i>A1, A2, A3</i>	Lateral aspect of ulna
<i>A.</i>	Anterior ligament
<i>AN.</i>	Annular band
<i>B1, B2, B3</i>	Posterior aspect of ulna
<i>C.</i>	Capsule of elbow joint
<i>D.</i>	The 'deep band' (continuation of <i>C.</i>)
<i>I.</i>	Posterior interosseous recurrent artery
<i>L.</i>	Lateral ligament of elbow joint
<i>P.</i>	Probe
<i>Post.</i>	Posterior part of annular ligament
<i>R.</i>	Rough ridge on ulna
<i>S.</i>	Supinator crest
<i>Sy.</i>	Reflexion of synovial membrane
<i>T.</i>	Special tubercle of ulna
<i>U.</i>	Ligament joining annular band from above

For convenience, all illustrations are shown as though taken from the right superior radio-ulnar joint.

AN EXPERIMENTAL STUDY OF PELVIC GROWTH IN THE RAT

BY T. J. HARRISON

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In a recent paper (Harrison, 1958), it was established in the rat from serial radiography following the insertion of marker pins, and from autoradiographs after a period of feeding with radioactive calcium, that a given point on the ilium moves caudally relative to the first piece of the sacrum during the growing period. The rate of caudal movement was shown to be approximately one-half of the rate of metaphyseal elongation below the iliac crest. This caudal migration of the ilium served to keep constant the ratio between the lengths of bone above and below the sacro-iliac joint, so preserving the general relations of sacrum and ilium, in spite of the observed fact that the cristal end of the bone grows five and a half times as fast as the acetabular end and continues to grow for a longer period. The mechanism of descent was not specifically investigated, but a study of the histology of the sacro-iliac joint region (Text-fig. 1) suggested that descent was brought about by the combination of metaphyseal elongation at the iliac crest tending to increase the distance of the crest above the sacrum, and tension in the superior sacro-iliac ligament stretching between the crest and the first piece of the sacrum tending to prevent this. If this is the correct explanation, then removal of the cristal growth cartilage, or section of the superior sacro-iliac ligament, should seriously interfere with iliac descent. On the other hand, if the ilium is pulled downwards by tension in structures attached below the sacro-iliac joint, then only the lower part should descend if the bone is transected just below the joint. These three operations were carried out on a series of young rats and this paper records the results observed.

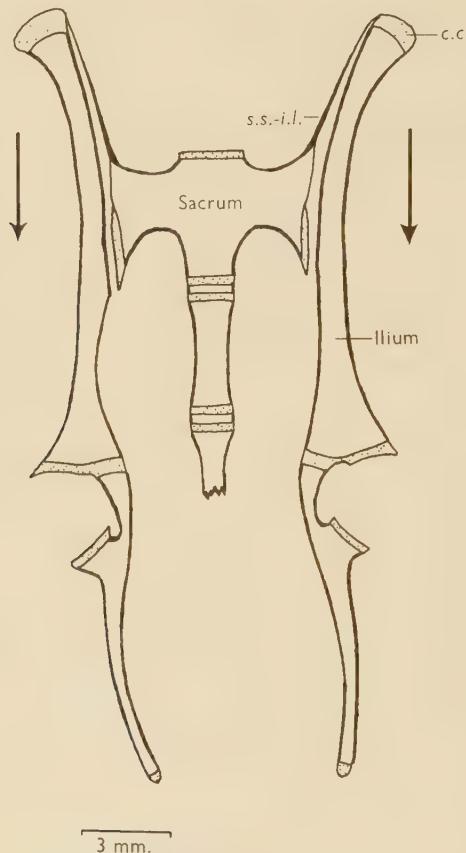
MATERIALS AND METHODS

In all, forty-three rats were operated upon and a further twenty-four litter-mates served as normal controls.

The iliac crest, including the growth cartilage, was removed unilaterally in ten rats varying in age between 3 and 32 days, and in two of these a steel marker was inserted into the damaged ilium at the time of operation. In a further three animals, 24 days old, bilateral removal of the iliac crests was performed.

The superior sacro-iliac ligament was incised in twenty-five animals between 9 and 27 days old. The operation was performed through a dorsal para-sagittal incision over the lumbo-sacral junction. The sacro-spinalis muscle was incised and retracted medially to gain access to the medial aspect of the ilium just above the level of the sacral ala, and the superior sacro-iliac ligament, which lies on this surface, was transected in a ventro-dorsal direction. The sacro-iliac joint was not dislocated or otherwise damaged at the operation.

In five 12-day-old animals the ilium on one side was completely divided between the sacro-iliac joint and the acetabulum and a part of the bone removed. An incision was made over the lateral aspect of the flank extending caudally from the ventral end of the iliac crest, the anterior edge of the tensor fasciae latae was identified and retracted dorsally, and the ilium was exposed in the cleft between the



Text-fig. 1. A coronal section through the pelvis of a 10-week-old rat to show the position of the growth cartilage on the iliac crest (*c.c.*), the attachments of the superior sacro-iliac ligament (*s.s.-i.l.*), and the direction of innominate bone migration.

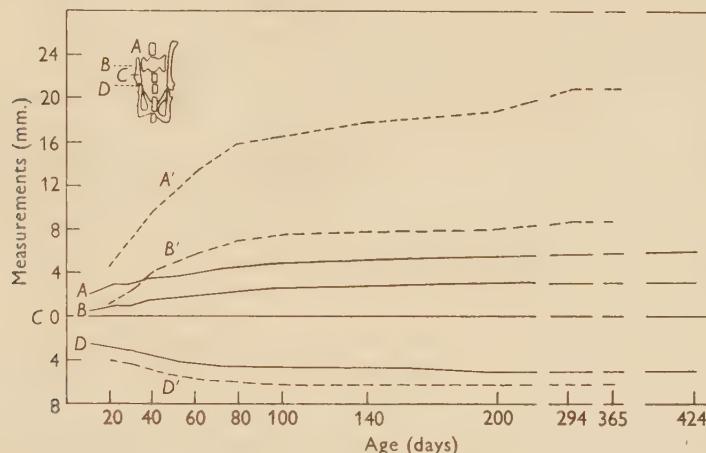
iliacus medially and the glutei laterally. Two or three mm. of bone between the sacrum and acetabulum were excised, completely separating the ilium into upper and lower fragments, and part of the gluteal musculature was detached from the upper fragment and inserted into the gap to prevent union.

Subsequent pelvic growth in each of these groups was studied by serial radiography of the living animals, on cleaned osteo-ligamentous preparations, and histologically in paraffin or celloidin serial sections. The radiographic and histological techniques used were similar to those described in previously reported pelvic growth studies (Harrison, 1958).

RESULTS

Removal of the iliac crest

Unilateral removal of the crest resulted in greatly retarded growth of the ilium, and increasing asymmetry of the pelvis, associated with greatly reduced caudal migration of the ilium on the operated side (Pl. 1, fig. 1*a-c*). Lumbar scoliosis concave to the operated side developed and became increasingly pronounced. Direct measurements on the radiographs of the animals in which pins had been inserted into the ilium showed that the growth rate at the crest was reduced from the normal $5\frac{1}{2}$ times to only $1\frac{1}{2}$ times the normal growth rate at the acetabular end, while caudal migration was reduced from the normal 7 mm. to approximately 2 mm. in similar 180-day growth periods (Text-fig. 2). However, the ratio of descent



Text-fig. 2. Growth curves derived from serial radiographs of two rats, one of which had its right iliac crest removed at 11 days of age. The continuous lines (*A*, *B*, *D*) show the vertical distance of the upper (*A*) and lower (*D*) ends of the ilium and the mid-point of the sacral ala (*B*) from the level of a marker pin (*C*) inserted into the ilium at the time of operation. The interrupted lines (*A'*, *B'*, *D'*) show corresponding levels in the control rat which simply had a marker placed in its ilium.

to crest increment was increased on the operated side from the normal 50% to between 65 and 70%. The pelvis became increasingly asymmetrical due to this unequal descent of the ilium on the two sides, which was shown, among other things, by greater obliquity of the superior pubic ramus on the operated side.

Histological examination showed that no regeneration of the cristal growth cartilage had occurred 10 weeks after operation, but instead the bone there was covered with a periosteal membrane beneath which there were signs of active intra-membranous ossification (Pl. 1, fig. 7). The superior sacro-iliac ligament, which was necessarily detached in removing the crest, had re-established connexion with the cristal periosteum. A surprising feature was the replacement of the normal fibrous-tissue-covered compact bone of the ilium opposite the sacro-iliac joint with cartilage-covered cancellous bone (Pl. 1, fig. 6).

Bilateral removal of the crests likewise retarded iliac growth and descent was greatly reduced on both sides. However, the pelvis remained symmetrical but was abnormal in shape, the sacrum being abnormally wide between the sacro-iliac joints, and the obliquity of the superior pubic ramus reduced, so that the symphysis pubis lay opposite the joint between the third and fourth pieces of the sacrum instead of opposite the first caudal vertebra (Pl. 1, figs. 4, 5).

Transection of the superior sacro-iliac ligament

Serial radiographs after sectioning the ligament showed that there was no interference with growth of the ilium, but its descent was reduced and increasing pelvic asymmetry developed as in the previous experiment (Pl. 1, fig. 3a, b). Histologically the sacro-iliac joint was normal in appearance after 20 days and the transected ligament at this time was partially repaired though the new fibrous tissue which bridged the defect had not the firm regular appearance of the original ligament. After 30 days abnormal changes began to appear in the upper part of the sacro-iliac joint. At first the ilium opposite the upper part of the sacral ala showed a medial bulge and then there was an increase in fibrous tissue between the two bones (Pl. 1, fig. 8). Ossification then extended into this fibrous tissue from the ilium, and where this came in contact with the sacrum fusion occurred so that by 52 days sacrum and ilium were firmly ankylosed (Pl. 1, fig. 9). Failure of descent of the ilium from this time onward must be attributed to this bony ankylosis rather than to transection of the superior sacro-iliac ligament, which in any case was fully repaired by this time.

Transection of the ilium below the sacro-iliac joint

Serial radiographs (Pl. 1, fig. 2) showed that the lower fragment failed to descend after section of the ilium, but that the upper fragment descended even faster than normal and increasingly overlapped the lower fragment on its outer side. Bony union did not occur between the fragments, the gap being filled with vascular fibrous tissue containing islands of cartilage. The asymmetry which developed in these experiments was different from that previously described in that it was confined to the pubo-ischial segment, the sacro-iliac part being relatively symmetrical.

DISCUSSION

These experiments show that removal of the crest of the ilium causes marked retardation of growth in this bone and seriously interferes with its caudal migration; and that transection of the superior sacro-iliac ligament causes a similar interference with iliac descent although this is later attributable to sacro-iliac ankylosis; but that transection of the ilium below the sacro-iliac joint is followed by accelerated descent of the upper fragment accompanied by failure of descent of the lower fragment.

Removal of the crest necessarily detaches the superior sacro-iliac ligament, but that failure of descent thereafter is due to absence of metaphyseal elongation, rather than to lack of ligamentous action, is shown by the fact that descent continues to be inhibited long after the ligament has re-established connexion with the upper end of the bone. In the second experiment bony ankylosis did not develop

until several weeks after operation, so this cannot be the explanation of the immediate failure of descent. It is evident therefore that either failure of metaphyseal elongation at the upper end of the ilium due to absence of the growth cartilage or transection of the ligament in the presence of an intact growth cartilage will inhibit descent. Further, the fact that the descent of the upper fragment of the ilium is accelerated after transection of the bone shows that descent is not due to traction applied below the joint. It follows that the action of the growth cartilage and the tension in the superior sacro-iliac ligament are both essential for normal descent and are the principal factors governing it. The hypothesis explaining the mechanism of descent put forward from a consideration of the mensural and histological studies previously reported thus receives experimental confirmation.

The importance of migration of the ilium for maintaining the general shape of the pelvis during growth has already been emphasized. The migration of the sacro-iliac joint, which is a necessary consequence of migration of the ilium, is facilitated (as was explained in a previous paper) by the unique structure of the joint, where the iliac surface is a smooth gliding plane without structural specialization or cartilage cover, and the shorter sacro-iliac ligamentous bands run in such a direction that they either do not hinder descent, or where likely to do so are successively removed from their attachment to the ilium by osteoclastic activity.

Abnormal changes in the sacro-iliac joint following either crest removal or ligamentous transection are difficult to explain. The formation of cancellous bone on the iliac side of the joint after cristal removal is probably due to altered stress across the joint surface consequent on pelvic asymmetry. New bone formation leading to ankylosis of the joint 7 weeks after ligamentous transection is probably also due to late-developing mechanical factors, rather than to the initial injury, for in the latter case one would expect new bone to begin to form within a very few days after the operation, but in fact the joint appeared histologically normal at least until 30 days afterwards.

A similar inequality in growth rates at the cristal and acetabular ends of the ilium has been shown in the pig (Payton, 1935) and man (Seigling, 1941), so that in these species also, and probably in mammals generally, caudal migration of the ilium brought about by ligamentous tension and metaphyseal growth is to be expected. Some recent marker-pin experiments have demonstrated that caudal migration of the ilium like that shown in the rat certainly occurs in the rabbit and guinea-pig also. Direct observation of iliac migration in man has not been reported, but the histology of the sacro-iliac region in a full-term foetus is so essentially like that of the rat that there is little doubt that the human pelvis possesses the descent mechanism and makes use of it.

This migration of one bone relative to another, involving shift of a diarthrodial joint, must be unique in vertebrate development; the only other examples of joint migration which may be cited are the displacements of the cranial sutures during growth, but these are not comparable. Finally, although caudal migration of the ilium is a unique phenomenon, it nevertheless falls into line with the principle that differential growth and movement are preferred to wholesale bone resorption as a means for preserving the shape and proportions of the skeleton while its parts are growing at different rates.

SUMMARY

The mechanism of caudal migration of the ilium in the rat during pelvic growth has been investigated. The operative procedures employed included removal of one or both iliac crests, with or without the insertion of marker pins in the damaged ilium; transection of the superior sacro-iliac ligament; and transection of the ilium caudal to the sacro-iliac joint. The growth changes were studied by serial radiography, the examination of gross preparations, and by histological methods.

Caudal migration of the ilium during growth has been shown to depend on the presence of an actively growing cartilage at the iliac crest and on the integrity of the superior sacro-iliac ligament connecting this cartilage to the sacral ala.

I wish to thank Prof. J. J. Pritchard for his helpful discussion and for his encouragement. I also wish to thank Mr W. T. Haddock for technical assistance and Mr G. R. Bryan for taking the photomicrographs. Finally, I am indebted to the Northern Ireland Hospitals Authority for a grant to purchase materials used in this investigation.

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EXPLANATION OF PLATE

Key to lettering: Il.=ilium; S.=ala of sacrum; L.=left side; R.=right side.

Fig. 1a–c. Serial radiographs of a rat's pelvis following removal of the left iliac crest when the animal was 10 days old: (a) 32 days, (b) 70 days, (c) 121 days after operation. $\times 1$.

Fig. 2. Radiograph of the pelvis of an 80-day-old rat on which transection of the right ilium had been carried out when the animal was 12 days old. $\times 1$.

Fig. 3a, b. Serial radiographs of a rat's pelvis following transection of the right superior sacro-iliac ligament: (a) 16 days, (b) 39 days after transection. $\times 1$.

Fig. 4. A ventral view of the pelvis of a 233-day-old rat in which both iliac crests had been removed when the animal was 24 days old. $\times 1.06$.

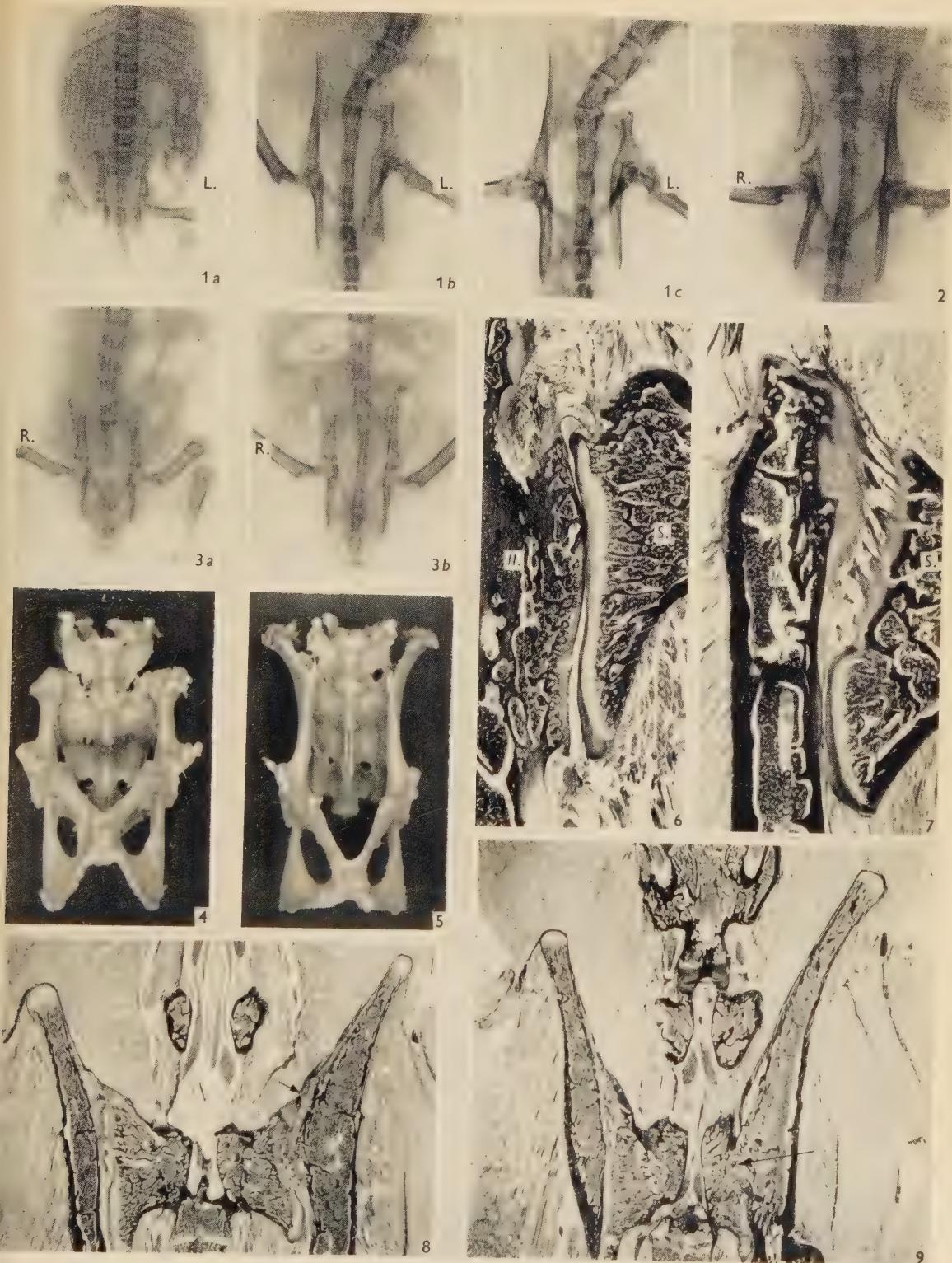
Fig. 5. A ventral view of the pelvis of a normal litter-mate of the animal whose pelvis is illustrated in fig. 4. $\times 1.06$.

Fig. 6. A coronal section through the sacro-iliac joint of a 94-day-old rat which had the iliac crest removed on this side when 10 days old. Haematoxylin and eosin. $\times 13$.

Fig. 7. A coronal section of the sacro-iliac joint in a 109-day-old rat in which the iliac crest had been removed at 32 days of age. Weigert's haematoxylin and van Gieson. $\times 12.8$.

Fig. 8. A coronal section through the sacro-iliac region of a 50-day-old rat which had the right superior sacro-iliac ligament transected 39 days previously. The arrow shows the region where transection took place. Weigert's haematoxylin and van Gieson. $\times 4.4$.

Fig. 9. A coronal section through the sacro-iliac region of a 62-day-old rat which had the right superior sacro-iliac ligament transected 52 days previously. The arrow points to the ankylosis of the sacro-iliac joint on this side. Weigert's haematoxylin and van Gieson. $\times 4.1$.



REVIEWS

An Introduction to Functional Anatomy. By D. SINCLAIR. (Pp. 426; text-figures 166. 42s.) Oxford: Blackwell Scientific Publications.

The danger of anatomy being presented in a purely descriptive way is, for medical students, happily no longer true. However, the class of reader for which Prof. Sinclair's book is intended—primarily occupational therapists, but others of the medical ancillaries also—is possibly less well catered for in this respect; if so this volume will certainly meet a need. Throughout its pages, whether tissues, systems or topography are being considered, structure and function are blended harmoniously, and with considerable discrimination, in a well-written text copiously illustrated by clear line-drawings. The style is pleasantly informal but the frequent use of homely analogies is overdone; the mild pleasantries which may enliven a lecture seldom reads well in cold print, and in places the impression is gained that the reader is being talked down to. This should be avoided in future editions.

Errors are few, but the following should be put right: on p. 112 Clarke's column is described as containing the motor cells of the sympathetic system; on p. 133 the proprioceptive pathway via the posterior columns of the cord and beyond is given as a two-neuronal system; on p. 168 the method of taking the blood pressure is misleading, implying as it does that the stethoscope is placed over the radial artery; on p. 273 the level of division of the trachea is indefinite owing to the omission of a word; on p. 299 proof-reading has been at fault and the paragraph below Fig. 108 should be transposed to the bottom of the page; in Fig. 141 the direction of the fibres of the interosseous membrane is incorrect.

All in all the book does very well what it sets out to do, and there seems little doubt that it will be a welcome addition to the libraries of our colleagues in the ancillary medical services.

E. W. WALLS

Morphologische Untersuchungen am Gehirn der Chiroptera (Mammalia). By R. SCHNEIDER. (Pp. 92; 78 figures and 11 tables of measurements.) Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft, 495. Frankfurt-am-Main. 1957.

This monograph is a useful account of the comparative morphology of the brain based on the examination of eighteen species of the Order Chiroptera (mega- and microchiroptera are included). It is limited to macroscopic features; some microphotographs are included, but the magnification is too low to give any significant information about the arrangement of cells or fibres.

The construction of lines between selected points (such as the anterior pole of the olfactory bulb, the mid-point of the convexity of the occipital pole, etc.) has enabled certain distances, angles and indices to be measured or calculated with a view to obtaining objective criteria for the assessment of morphological features like the degree of projection of the temporal pole. While these quantitative data may be of value in making comparisons within the Order under investigation, there is some doubt whether they form a useful basis for comparison with other mammalian brains. Of greater significance are the comparative measurements of the surface area of the olfactory bulbs, the palaeo- and neopallium and the hippocampal formation. No systematic relationship between these measurements and the taxonomic position or size (crown-rump length) of the animal was found. It is also concluded that the relative increase of the neocortical area found in some species is not a result of allometric growth.

Other sections of this monograph deal with the mid-brain, hind-brain and cerebellum and with the relationship of the brain to the meninges and skull, but no further quantitative

data are given. The main conclusions, that the brains of the Macrochiroptera diverge least from the conditions found in a generalized insectivore, and that in the Microchiroptera specializations, often limited to a particular species, are very marked, some of them related to the development of the acoustic apparatus, are not strikingly new. The monograph is essentially an accurate record of many descriptive features of the brain in a particularly interesting group of animals. It will be of considerable value to anyone working in this field, or with interests in the comparative morphology of the primitive mammalian brain.

F. GOLDBY

Handbuch der mikroskopischen Anatomie des Menschen. By W. v. MÖLLENDORFF. Fortgeführt von W. Bargmann. Bd III. *Haut und Sinnesorgane.* Teil 3: *Die Haut. Die Milchdrüse.* By E. HORSTMAN and A. DABELOW, 1957. (Pp. viii + 324, 359 illustrations, many in colour. Ladenpreis: DM. 198; ganzleinen, DM. 216). Bd IV: Teil 5: *Mikroskopische Anatomie des vegetativen Nervensystems.* Ergänzung zu Bd IV/1. By P. L. STÖHR, Jr. 1957. (Pp. xi + 678, 501 illustrations, many in colour. Ladenpreis; DM. 240; Halbfranz. DM. 258). Berlin; Göttingen, Heidelberg: Springer-Verlag.

These two excellently printed and produced, but very expensive, volumes continue the monumental and encyclopaedic von Möllendorff *Handbuch*. The section on the skin is by Prof. Ernst Horstmann, of Kiel. In 236 pages he brings up to date the advances in our knowledge of the histology of the skin since Hoepke's original contribution to the series in 1927. Although sense organs are included in the title of this section there are, in fact, only six pages concerned with cutaneous innervation. The section on the mammary gland is by Dr Adolf Dabelow, of Mainz. Each of these sections is beautifully illustrated. Nevertheless, and in spite of a short addendum on the electron microscopy of skin by Horstmann, more electron micrographs could have been expected in a volume the intent of which is, presumably, to present current knowledge as revealed by recent techniques. Possibly, too, there is an excess of drawings amongst the illustrations. The coverage of the related literature, however, is admirable. There are some 1600 references appended to the skin section, and nearly a 1000 to that on the mammary glands. All relevant and significant British and American contributions of the post-war period seem to have been included in the bibliographies and to be referred to in the text.

Prof. Stöhr's volume on the vegetative nervous system can only be described as monumental. It will be invaluable to any student of the autonomic nervous system. The bibliography alone includes some 3500 references since 1928, and no gaps in this coverage of the world's literature have made themselves obvious. Not everyone will agree with all of Prof. Stöhr's interpretations. More particularly those relating to the termination of autonomic fibres will not convince all anatomists that finality has been reached. Over-dependence on Bielschowsky's technique as method and on drawings for illustrations leaves an old problem with a not altogether satisfying answer. But against the magnificent contribution which the volume, as a whole, represents, any criticism is bound to seem carping. It is most pleasant to note Prof. Stöhr's dedication—'dem altehrwürdigen Oriel College in Oxford gewidmet'. This friendly gesture and the careful attention paid by Prof. Stöhr, in his review of the literature, to our *Journal* and *Proceedings* will be widely appreciated by members of the Anatomical Society of Great Britain and Ireland.

J. D. BOYD

BOOKS RECEIVED

The Tissues of the Body. An Introduction to the Study of Anatomy. By W. E. LE GROS CLARK, F.R.S. 4th edition, 1958. (Pp. xi+415. 40s.) Oxford: Clarendon Press.

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Human Anatomy and Physiology. By N. D. MILLARD, B. G. KING and M. J. SHOWERS. 4th edition, 1956. (Pp. vii+593. 35s.) Philadelphia and London: W. B. Saunders Co.

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THE EARLY DEVELOPMENT OF THE
BRAIN IN MARSUPIALS
PRELIMINARY COMMUNICATION

BY PROFESSOR J. P. HILL AND DR K. M. WATSON

(*A paper read to a meeting of the Anatomical Society of Great Britain
and Ireland on 19 November 1920*)

FOREWORD

Prof. J. P. Hill died in May 1954, leaving behind him a large mass of important scientific manuscripts and papers (with very many associated drawings). These are now being subjected to scrutiny and collation, and it is expected that most of them will sooner or later be published. It is difficult to forecast how long this will take, but it will certainly be some years. The present paper was read before the Anatomical Society some thirty-seven years ago at a meeting held at University College London on Friday 19 November 1920. It has never been published because at that time and for some time previous, due to war conditions, the Society had been unable to print any proceedings. No attempt to publish it has been made since.

Its reading brought forth energetic criticism, particularly from those who felt that the germ-layer theory was in danger. Nevertheless, even at this late hour it is felt that this preliminary communication should be published since (*a*) it shows that the authors in 1920 were able to provide evidence based on purely morphological grounds of the tremendous plasticity of the structure called the neural crest confirmed and extended in more recent years by other investigators using experimental methods (Hörstadius, *The Neural Crest*, Oxford University Press, 1950), (*b*) it records the only evidence available as to the history of the neural crest in marsupials and forms a necessary preliminary to the very extensive work on the neural crest in this group undertaken by Prof. Hill and Dr K. M. Watson and which still awaits publication.

T. THOMSON FLYNN

The work on which the following communication is based was begun at the end of 1911. After making graphic reconstructions from serial sections of *Dasyurus* stages, I became convinced that a large part of the mesenchyme of the maxillary and mandibular arches was derived from the neural crest proliferations. Prof. Hill accepted this conclusion with a reluctance which was due in large part, I think, to his adherence to the germ-layer theory.

Prof. Elliot Smith, who took an interest in the work throughout, repeatedly urged Prof. Hill to publish a brief note on the subject of 'ecto-mesoderm' without waiting to complete the work on the development of the brain, and this short paper was the delayed result. It is unfortunate that it was not published at the time and, indeed, it is only by the good work of Prof. Flynn that it has been found and published now. I had no part in the production of this communication and, for reasons now forgotten, I was not even at the meeting of the Anatomical Society when it was presented.

KATHARINE M. WATSON

For some years past the authors have been engaged in studying the early development of the nervous system in the marsupial embryos in the collection of one of us, and now that our work is approaching completion we have thought that a demonstration of some of our results might be of interest to members of the Society. The investigation (unduly prolonged by various unavoidable and more or less lengthy

interruptions) has proved laborious and tedious in the extreme. Although we have no strikingly new results to record, we venture to hope that our work may do something to clarify, if not to correct, current views on the ontogenesis of the vertebrate nervous system. I think that those of you who are familiar with the literature will readily agree that current views stand in need of considerable clarification.

The material at our disposal consists of a considerable number of early embryos of various genera of Marsupials including *Dasyurus*, *Perameles*, *Didelphys*, *Macropus* and *Petrogale*, mostly in an excellent state of preservation and forming when dovetailed together a very fair series, but *unfortunately* of no one species do we possess anything approaching a continuous series. We say unfortunately because one of the things which has impressed us very forcibly during our work is that the development, more especially of the peripheral nervous system, can only be adequately followed in a material comprising a perfectly continuous series of embryos from the first appearance of the neural plate onwards. Although the material at our command comes nowhere near that ideal, in certain other respects it presents decided advantages; in particular, in the marsupial embryo, closure of the brain-plate is for some reason, perhaps connected with the expansion of the fluid-filled blastocyst, long delayed so that events which in the monodelphian embryo occur in the dark so to speak, after closure is more or less complete, take place here in the open, uncomplicated by the process of closure. This late closure is of great value in the study of the first origin of the peripheral nervous system.

Our work falls naturally into two divisions: (1) the early development of the peripheral nervous system, in particular of the cranial ganglia, (2) the early development of the brain. In selecting our material for this demonstration we found it quite impossible to deal adequately with both of these divisions of our subject in the time at our disposal, so we have thought it best to limit ourselves on this occasion to a demonstration of our observations on the development of the peripheral system. Thanks to the skill of our technical assistant, Mr Pittock, we have at our disposal a fine series of lantern slides, many of them made from photomicrographs, and as our primary object in occupying your time to-day is to show you something of our material I want to make this talk as brief as possible, and for the same reason I shall not attempt to deal in any detail with the history of this huge subject. Notwithstanding our Secretary's remarks on the Agenda paper we make no claim that our conclusions are new, although as a matter of fact we thought that some of them were so, before we had waded through the huge and extremely verbose paper, valuable as to the development of the cranial nerves in *Gallus*, by Belogolowy published in 1910.

In that paper Belogolowy distinguished *two* sources of origin of the neural crest:

(a) The first formed part of the crest, he says, is derived by proliferation from the strip of thickened ectoderm which intervenes on each side between the margin of the neural ectoderm and the general ectoderm. These strips have long been known. They first received definite recognition in 1878 from Marshall. He observed them just after they had begun to proliferate so as to form longitudinal ridges occupying the angle between the closing neural tube and the general ectoderm. Marshall termed them the 'neural ridges' and he showed that when the neural tube does close, the two ridges unite in the middle line to form a band overlying the neural tube. This

median band he called (1879) the 'neural crest'. It must be noted in passing that this term is frequently used nowadays in a somewhat different sense to include the cells proliferated from the neural crest. My old friend and teacher John Beard in 1888 emphasized that these proliferating strips (the neural ridges of Marshall) lie entirely outside the limits of the definitive neural plate, that in fact they are extra-neuronal, but he made the mistake, as we believe, of maintaining that these extra-neuronal strips exist also in the spinal-cord region and that they furnish the spinal ganglia.

In our early marsupials homologous extra-neuronal strips of thickened and eventually proliferating ectoderm are easily recognizable, outside the limit of the flat brain-plate. Long before we read Belogolowy we had designated these strips as the paraneuronal ectoderm and the cells to which they give origin we propose to term the paraneuronal (or paramedullary) proliferations or crests. It is the same thing as the mesectoderm of Miss Platt (1894, 1898) and the ecto-mesoderm of A. Goette (1874-5). The paraneuronal strips really form in our opinion a pair of marginal placodes, each of which extends from about the level of the optic grooves back almost to the level of the first pair of mesodermal somites. *They do not extend to the trunk.*

(b) Then a second source of origin of the neural crest cells, according to Belogolowy, is to be found in the neural tube itself since cells are directly proliferated from its dorsal surface. These cells pass down along the sides of the tube (as Beard in 1888 had already pointed out), unlike the cells of the first or extra-neuronal proliferation which tend to spread out below the ectoderm. We had also independently observed this second source of origin in our embryos and we propose to speak of it, until we find a better name, as the ecto-neural or neuro-ectodermal proliferation or crest (=medullary crest).

Belogolowy states that from the metaotic region backwards this ecto-neural proliferation gradually increases in size and completely replaces the paraneuronal as we have termed it. Behind the ear the paraneuronal proliferations according to him occur only in the occipital region. In our view these are confined to the anterior part of that region in front of the first somitic clefts.

There is of course yet another source of cranial ganglionic neuroblasts, and that is from the localized thickenings of the cephalic ectoderm which we now, following von Kupffer, consistently speak of as *placodes* and which are developed in relation to the ganglion of the V, VII, IX and X cranial nerves. Such placodes are easily recognizable in our own material and there can be no doubt from the work of Held (1909), Landacre (1910) and others that they do actually contribute neuroblasts to the cranial ganglia in lower vertebrates.

We have then these three possible sources of cranial ganglionic neuroblasts: (1) the paraneuronal or marginal placodal, (2) the ecto-neural, (3) the epibranchial placodal, as contrasted with a single ecto-neural source for the spinal ganglionic neuroblasts. Of these three possible sources, we personally have been most interested in the paraneuronal since the paraneuronal proliferations form such conspicuous lobe-like masses in the cephalic region of our early marsupials.

Selenka (1886-7) was the first to observe in embryos of *Didelphys* what we now term the anterior lobes. He termed them the 'Head Plates' of mesoderm. Then C. J. Martin and one of us (J. P. H.) observed the same lobes in an early embryo of

Ornithorhynchus and following Selenka's lead these two authors also regarded them as mesodermal cephalic lobes (see Hill & Martin, 1894, pp. 45–57). Still later (1907) J. T. Wilson and one of us (J.P.H.) in a paper dealing with the early development of *Ornithorhynchus* described in some detail four embryos (including the Martin embryo) in what they termed the neurular stage of development (characterized by the flat neural plate) and by the presence alongside it of what they regarded as the primary ganglionic primordia. In particular, Wilson & Hill interpreted the cephalic ('head') plates of Martin & Hill situated at the sides of the anterior half of the brain-plate as of neural crest origin and as being in reality the trigeminal ganglionic primordia. This interpretation they extended to the corresponding plates in marsupial embryos.

After prolonged study of these and other embryos Mrs Watson and I are not now inclined to accept that interpretation unreservedly. We believe that the paraneural proliferations which form the so-called anterior lobes are ecto-mesodermal in the sense that part of their constituent cellular elements is neuroblastic in significance and destined to be incorporated in the trigeminal ganglion, whilst the remaining and larger part of these lobes furnishes the bulk of the mesenchyme of the mandibular arches.

Finally, as regards the development of the individual cranial ganglia, our observations appear to justify the following conclusions:

(1) *Trigeminus*. The ophthalmic ganglion is probably partly of paraneural, partly of placodal origin. The main mass of the Gasserian (maxillo-mandibular) ganglion is derived from all three sources, the ectoneural predominating.

(2) *Facialis*. The ganglion of the facial is formed by a mixed paraneural and ectoneural contribution with additions from an ectodermal as well as an entodermal placode.

(3) *Glossopharyngeal vagus complex*. This is primarily of paraneural derivation with a placodal contribution and a small ectoneural constituent forming the so-called *root ganglion*.

(4) The root ganglia of the cranial nerves are serially homologous with the dorsal root ganglia of the spinal nerves.

If we were speculatively inclined, which we are not, we should suggest that the primary optic pit represents the anterior segment of our paraneural strip which has become secondarily included in the brain-plate and that the auditory plate is a derivative of its posterior region.

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THE FACTORS CONTROLLING THE DEVELOPMENT
OF THE DORSAL ROOT GANGLIA AND VENTRAL
HORN IN *XENOPUS LAEVIS* (DAUD.)

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INTRODUCTION

The famous essay on the problems and scope of '*Entwicklungsmechanik*', with which Wilhelm Roux (1895) began his *Archiv* contains the definition of his two concepts of 'self-differentiation' and 'dependent-differentiation'. Much of the subsequent development of the subject, as Roux foretold, has been concerned with distinguishing between the operation of these two groups of 'complex components'. Moreover, his dictum that 'self-differentiation and dependent differentiation may occur in the most varied combination either simultaneously or successively' (Wheeler trans. 1895, p. 165) has more than once been confirmed. Thus, for instance, Stern (1955), in a review of the present evidence on the means by which the various genes for eye colour in *Drosophila* receive expression during development, concludes that 'there is no general rule as to when dependent and when autonomous differentiation may be expected'.

In only a few directions has the experimental analysis of development advanced sufficiently to reach such generalized conclusions. The study of the development of the structure of the central nervous system of the vertebrate embryo is still far from this point, but here instances of both self- and dependent-differentiation have been recognized. For most investigators in this field, the spinal cord has offered the main promise of successful analysis, and here much of what is known concerns the trophic influence of the limbs on the cord at brachial and lumbar levels. Researches on several groups of tetrapods have shown that in these regions, the size of a dorsal root ganglion, in terms of the number of its component cell-bodies, depends upon the presence of the limb innervated by their fibres (Piatt, 1948). On the motor side also, in forms where a ventral horn is readily distinguishable, its size is also governed by peripheral influences (Hamburger & Keefe, 1944). At the same time, however, transplantation of the segments of the cord of the early chick embryo (Wenger, 1951) has revealed the existence of self-differentiating factors within the cord. Thus only the cord from the normal limb-levels is able to react to the presence of the peripheral field by developing a ventral horn.

For the dorsal root ganglia, comparison at different levels under normal and experimental conditions has generally been confined to such data as the total number of cells, the mitotic index, and the proportion undergoing degeneration at each stage. The present study is concerned in the first place with differences in the size of the component perikarya within the ganglia at various levels*. In the larva

* In *Xenopus*, as in the frog (Gaupp, 1896, pp. 156, 166) the first spinal nerve is rudimentary, and the ventral root of the second is the hypoglossal nerve. The first spinal ganglion belongs to S3. The forelimb is innervated by spinal nerves S3 and S4, and the hind-limb by S8, S9 and S10. S7 occasionally contributes to the lumbar plexus.

of *Xenopus laevis*, soon after the dorsal root ganglia are first recognizable, a clear distinction emerges between those at limb levels and the remaining ganglia elsewhere in the cord, for within the latter are to be found some perikarya of relatively large size; such, however, are not found at brachial and lumbar levels. Such differences have already been described in *Rana pipiens* by Taylor (1943). As soon as the spinal ganglia are recognizable, each is seen to contain a group of large cells, about twenty in number. The remainder of the ganglion consists of smaller cells, which at lumbar levels (ganglia S8, S9, S10) increase rapidly in number and here soon make up the vast majority of the constituent cells. There is a similar difference between the diameter of the fibres which belong respectively to these two groups of cells; Taylor observed that only fine fibres coming from small cells enter the limb buds. In later stages, progressive growth of these sensory neurones in both *Rana* and *Xenopus* blurs the distinction in size between the two original groups of primary and secondary cells.

In *Xenopus*, at mid-larval stages the distinction between the spinal ganglia at limb and non-limb levels is carried to a further stage, for in this Anuran, those at brachial and lumbar levels contain none of the larger cells which are found elsewhere in trunk and tail segments. The distinction between the two types of spinal ganglia is recognizable early in their development and it seemed that this circumstance provided an opportunity for a fresh study of the factors which control the differentiation of the spinal ganglia. Accordingly, our experiments have been made primarily in order to test the influence of various operative procedures on the composition of the dorsal root ganglia at lumbar levels in larval *Xenopus*. During the course of this work, we have also learnt something of the factors which govern the development of the ventral horn within the cord. In this paper we describe these investigations and discuss the evidence for both self-organization and dependent differentiation in the cord and dorsal root ganglia of this form.

It is first desirable, however, to describe the normal differentiation of the ganglia, and to indicate certain features in the early development of the hind-limb into which their afferent fibres will penetrate.

MATERIAL AND METHODS

Ovulation and amplexus in adult *Xenopus* were induced by chorionic gonadotrophin by techniques to which reference is made in a previous paper (Tschemi, 1957), where the culture of the resulting larvae is also briefly outlined.

The details of each type of operation on the experimental tadpoles will be described under separate headings. Here, however, certain general features of procedure may be mentioned. The animals were first narcotized in a 1:7000 solution of the anaesthetic MS 222 (Rothlin, 1932). They were then transferred to a small Petri dish, the floor of which had first been coated with agar. During the operation, the dish was filled with a solution of MS 222 in 0·6% NaCl, at a concentration of either 1:7000 or 1:14,000. The animal was held in position either by fine glass rods or strips of tantalum foil pushed into the agar base. Young larvae can be left in this position after the operation still anaesthetized, where it is necessary to prevent movement until two apposed tissue surfaces have united. Finally, the operated animals were

placed in tap water; larvae which had only recently hatched were first immersed in Holtfreter's solution, either at full strength or in various dilutions. When healing was complete, the animals were transferred to tap water.

When tadpoles had reached a suitable stage of development, they were fixed in Bouin's solution and the tail was immediately severed. This step is necessary to avoid collapse of the notochord and consequent distortion of the tissues (Hughes, 1957). After a period of at least 24 hr. in fixative, they were then dehydrated and, where necessary, decalcified in acidified 70 % alcohol. They were embedded in paraffin and the blocks were sectioned at an appropriate thickness. The sections were stained either in Ehrlich's haematoxylin and eosin, or were silvered by Bodian's protargol method.

Simplified reconstructions were plotted on squared paper for all section series which were studied in detail, in the first place to identify the individual members of the series of spinal ganglia. The total number of cells within a ganglion was counted through the relevant sections under a magnification of $\times 500$. Enumeration was much aided by a squared grid within the eyepiece of the microscope. In order to minimize the errors which arise from the inclusion of a cell within adjacent sections, counting was restricted to these cells where the nucleolus was present within the section. It is very rare that the nucleus of a differentiated neuron in these ganglia has more than one nucleolus; these bodies in most neurones are less than 2μ in diameter. The nucleolus is much bigger in the largest neurones, but these cells are present only in small numbers, and can be followed individually through adjacent sections.

The early stages of normal development of the hind-limb of Xenopus

Tschernoff (1907) and Taylor (1943) have described the normal development of the hind-limb in *Rana pipiens*. In this Anuran, the hind-limbs originate from a specialized area of the body-wall immediately on either side of the ventral fin. The ectoderm in this area is thickened, as is also the corresponding segment of coelomic wall beneath. Between the two thickened epithelia, a dense mesenchyme accumulates (Filatow, 1930, 1933). The mesoderm of the limb originates in the first place from this specialized region of the somatopleure, and later migrates outwards into the limb bud. Recently, Saunders (1948) has shown that in the chick, an apical cap in the ectoderm of the limb bud causes the mesenchyme immediately beneath to proliferate and thus to lay down the presumptive skeletogenous material in a proximo-distal order; Tschumi (1956, 1957) has demonstrated that the same principle holds in the growth of the hind-limbs in *Xenopus*.

In the early stages of development of the latter, the specialized region of somatopleure, the thickened ectoderm and the dense mesenchyme between, are all recognizable, though with certain differences from their condition in *Rana*. In *Xenopus* at the relatively early stage of 6 mm., the specialized region of somatopleure begins to differentiate into a layer of myoblasts (Pl. 1, fig. 2). Any contribution to the limb from the coelomic wall can come only before this period. A little earlier, at 5.7 mm., these myoblasts have not yet differentiated, and the mesenchyme around the anal tube is uniformly dense (Pl. 1, fig. 1). No indication of the limits of the future limb area is then recognizable.

At 6.5 mm. the future limb mesenchyme is clearly delimited. From then it has the form of a cone of cells facing inwards, with the apex in contact with the muscle layer already described. These cones are situated immediately caudal to the hinder limit of the coelomic cavity. The ectoderm of the limb area is already thicker than elsewhere; by 9 mm. in this region there are two layers, the inner of which is the deeper (Pl. 1, fig. 3). Here the nuclei are rounded, in contrast to the flattened nuclei of the upper layer. A day later, at 9.2 mm., mitotic figures are numerous throughout the limb area. They remain conspicuous during the further growth of the limb. From the outside, the limb bud becomes recognizable at about 12.5 mm., and as Nieuwkoop & Faber (1956) show, it attains a height equal to its diameter at about 15 mm.

A second point of difference between the development of the hind-limbs in *Rana* and *Xenopus* is that whereas in the former the axis of the limb buds come to lie parallel to the median plane of the larva, in *Xenopus* the early limb buds from their first appearance point straight outwards and nearly at right angles to the median plane.

The first entry of nerve fibres in the hind-limb buds

In section series stained by Bodian's method, the first nerve fibres in the neighbourhood of the future limb buds are seen on the medial side of the somatopleuric muscle band which has already been described. The earliest stage at which we have been able to recognize fine fibres in this position is at 10.3 mm. Their distribution at 16 mm. is still restricted to the medial side of the muscle fibres. By 18 mm., however (stage 49 of Nieuwkoop & Faber), some fibres are beginning to grow laterally into the mesenchyme of the limb bud (Pl. 1, fig. 4); after motor twigs to the muscle fibres are given off, the main nerve bundle extends ventrally below the somatopleuric muscle and then turns to enter the limb mesenchyme, where it fans broadly outwards. Fibres both to limb bud and muscle are then equally fine in calibre. By 20 mm., the limb nerve is recognizable even without the aid of silver impregnation.

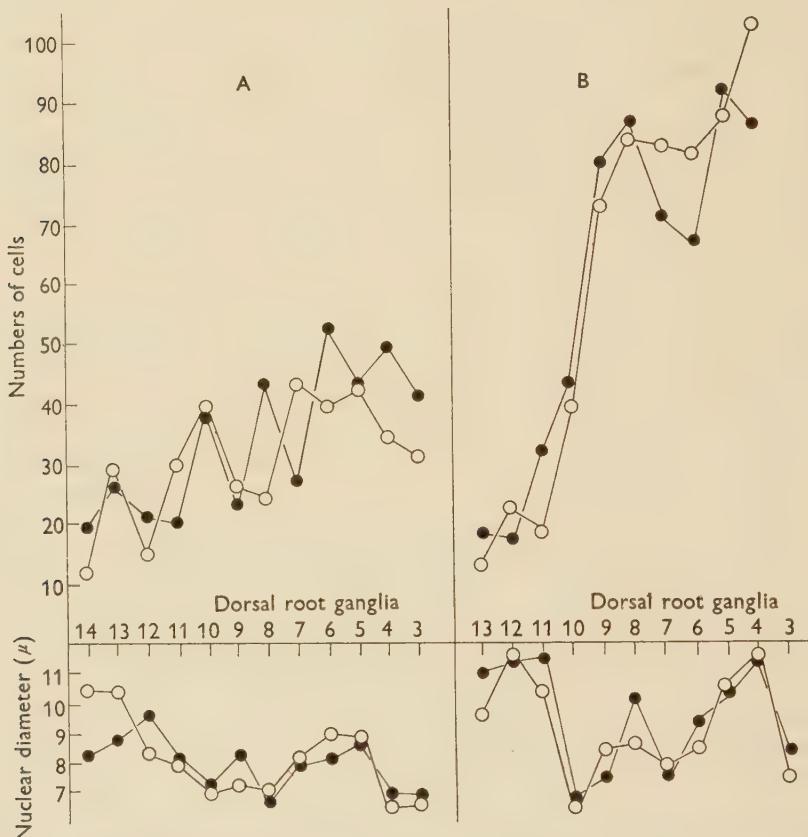
*The development of the dorsal root ganglia in *Xenopus**

Some account of the development of these ganglia has been given both by Nieuwkoop & Faber (1956) and by Hughes (1957). The former authors noticed that the neurones which compose the ganglia were of various sizes. They first recognized the dorsal root ganglia at stage 39 (6.0 mm.), when each consists of about 6–10 neuroblasts. By stage 44 (8 mm.) the number of cells in each ganglion has increased by threefold. Marked diversity in size of these cells is first seen in the tail, at stage 46 (9–12 mm.); this is true also of the ganglia at trunk levels by the succeeding stage (12–15 mm.).

In the tail, the dorsal root ganglia are so diffuse that the boundary between adjacent ganglia cannot always be recognized with certainty. A further peculiarity of these ganglia is that their constituent cells are divided into two groups, one close to the cord within its pigmented meningeal sheath, and the other ventro-lateral to the first, between the notochord and the inner surface of the myotome. This part of a tail ganglion consists of its larger neurones, which are often fewer than ten in number.

Text-figs. 1, 2 are present data on the number and size of the neurones of the dorsal root ganglia at three stages of normal development. Counts were made of

the total number of cells in each ganglion from the third, the most cranial of the series, to the thirteenth, or beyond. At relatively early stages these figures include not only neuroblasts, but also some future Schwann cells; later it becomes progressively easier to distinguish between these two categories of cells derived from the neural crest. The absolute numbers in these counts, however, is of less importance than the comparison of the ganglia at different levels in the same larva.

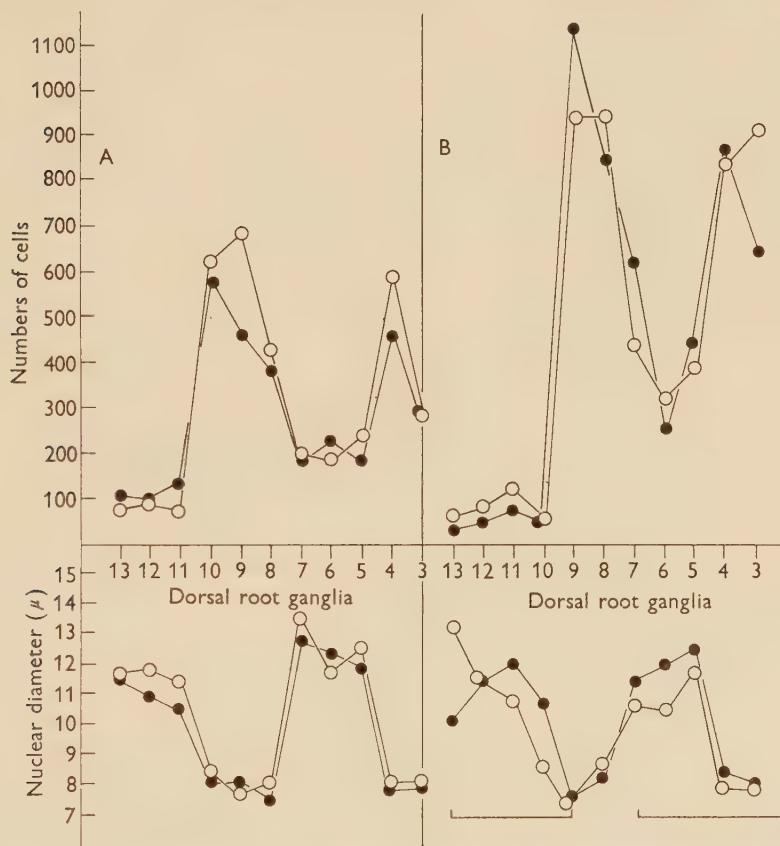


Text-fig. 1. Numbers of cells in spinal ganglia on each side (above) and the diameter of the largest nucleus of each ganglion (below). A: normal tadpole at 11.0 mm.; B: normal tadpole at 14.7 mm.

In order to show the sizes of the neuroblasts within these ganglia, the diameters of the nuclei of the largest cells were measured with an eyepiece micrometer. Usually, the nuclear membrane is oval rather than circular in outline, and so the mean of the diameters along major and minor axes was calculated. For two ganglia, a complete survey was made of nuclear diameter for every cell, and the results are expressed as histograms in Text-fig. 3.

These measurements were plotted as cumulative percentages on probability paper, in the manner described by Harding (1949), in order to determine whether these data conform to any definite type of distribution. This method has already

been applied to the study of nuclear diameter in the mammalian liver by Hughes (1952). In the present instances, both in the small-celled limb ganglion and the non-limb ganglion with its larger perikarya, the distribution of nuclear diameters was unimodal in type. This suggests that the neurones of the dorsal root ganglia form



Text-fig. 2. Numbers of cells in spinal ganglia on each side and the diameter of the largest nucleus of each ganglion. A: normal tadpole at 29 mm. (Text-fig. 8); B: experimental tadpole (CSL 21.5). The longitudinal extent of the cleft is indicated below.

a homogeneous race of cells and that their individual differences in size are not due to any distinction between discrete categories of cells such as is caused by heteroploidy.

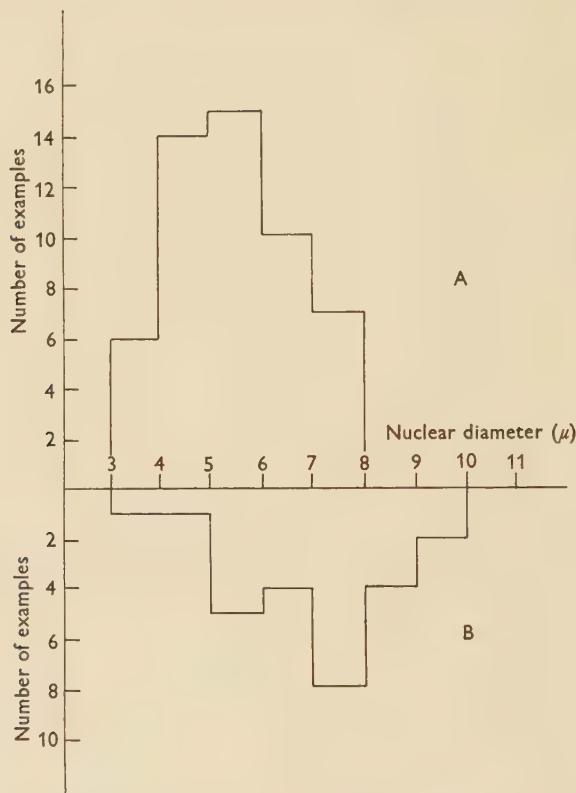
In general, the distinction between large and small neurones in the dorsal root ganglia is obvious without actual measurement, mainly because the large neurones have an obvious cytoplasm, in which the nucleus lies to one side. The small neurones are closely bunched together, and only towards the end of the larval period can their cytoplasm be separately distinguished. For this reason nuclear rather than cell diameter has been chosen for measurement, although this itself is not the most obvious difference between the two categories of neurones.

Text-figs. 1 and 2 demonstrate these main points:

(1) There is a gradient along the body axis with respect to cell number in the dorsal root ganglia, which at first bears no relation to the limbs (Text-fig. 1A). Later

superimposed on this are two peaks at brachial and lumbar levels, generally with maxima at S4 and S9 respectively (Text-figs. 1B, 2A).

(2) A distinction in the maximum nuclear diameter between ganglia at limb and non-limb levels gradually becomes recognizable before the brachial and lumbar



Text-fig. 3. Histogram of nuclear diameter for spinal ganglia S10 (A) and S11 (B) in normal tadpole at 14.7 mm. (The same as in Text-fig. 1B.)

peaks in total numbers of cells have appeared (Text-fig. 1A, 1B). This distinction, however, becomes progressively sharper towards the middle period of larval life, when the two sets of data henceforward follow inverse relationships.

(3) The differences between the ganglia at limb and non-limb levels, both in respect to cell size and cell number, are established before the entry of nerve fibres into the limb buds.

EXPERIMENTAL RESULTS

(1) Removal of the early limb buds of one side (Series HLRF)

In the first set of experiments a hind-limb bud was removed from each of a series of larvae soon after this rudiment was first recognizable, at stages 49–51 of Nieuwkoop & Faber (1956). These tadpoles then continued their development and individuals were fixed at timed intervals after the operation.

In order to promote the complete extirpation of the limb field, and to hinder any subsequent regeneration, the surrounding tissues were also removed. These included part of the abdominal wall and peritoneum, and also the ventral portions of the adjacent myotomes, together with part of the tail fin. For the operation, two pairs of fine watchmaker's forceps were used. The incisions round the limb area were made by first pricking one blade of the first pair through the whole depth of tissue to be removed. The two blades were then firmly apposed, and the resulting cut through the tough epidermal layer was then continued by means of the second pair of forceps. Special care was necessary to avoid injury to the hind-gut in the region of the operation. The opening of the abdominal cavity does not cause any appreciable mortality. In these experiments the gap healed within two days. Immediately after the operation, the tadpoles were transferred to tap water.

Table 1. Cell counts in ganglia S6 to S11 after limb ablation on left side (Series HLRF)

Days after operation	Length (mm.)	Side	Spinal ganglia					
			6	7	8	9	10	11
1	19	R.	69	73	76	76	61	51
		L.	69	70	60	92	74	33
4	20	R.	135	147	158	169	196	87
		L.	128	103	128	193	189	52
6	21	R.	116	140	179	189	163	86
		L.	143	159	135	132	143	71
9	22.8	R.	184	304	380	516	227	149
		L.	175	222	240	270	232	93
11	24	R.	267	163	241	369	253	111
		L.	194	163	210	297	249	109
18	39	R.	355	222	535	783	507	122
		L.	301	132	372	415	300	126

When sections were finally prepared from these experimental larvae, the cells composing spinal ganglia 6–11 were counted on both operated and unoperated sides. The data from this experiment are given in Table 1. These results indicate that in *Xenopus*, as in other vertebrates on which similar experiments have been performed, the growing limb influences the rate of increase in cell number within the dorsal root ganglia by which it is innervated, and that removal of a limb results in a slower growth of the ganglia on the operated side.

Usually, however, in such investigations the aim has been to record the final difference in size in this respect between the two sides of the animal, which has generally not been fixed until the end of its growth period was approaching. Such differences are usually larger than those in Table 1, for here we wished primarily to see how early this differential growth could be recognized.

Table 1 suggests that as early as 6 days after the operation the spinal ganglia S8 and S9 are already growing more slowly on the operated side. Later, this slackening spreads to ganglion S10 and also to S6 and S7. The two latter may be influenced by the removal of tissue surrounding the limb area at the time of operation. Throughout this series of experiments the first tail ganglion S11 is unaffected by extirpation of the limb. In one larva, namely that fixed 9 days after operation, S7 was found to be small-celled on both sides and to send fibres into the limb on the

unoperated side. Here, moreover, a differential growth on the two sides seems to have been established earlier than in other larvae where this ganglion does not contribute to the hind-limb plexus.

In none of these series was it possible to recognize any distinction in size of the neurones of the ganglia on the two sides. In all of these experimental larvae, the limb buds had been removed when the rudiment had only recently appeared on the surface. This stage corresponds to Nieuwkoop & Faber's No. 48, when the larva is 15 mm. in length. At this time, as Text-fig. 1B shows, the distinction in size between the neurones of the ganglia at limb and non-limb levels is already established.

This first series of ablation experiments thus leave unanswered the question whether any peripheral influence on the development of the dorsal root ganglia is exerted in the first phase of the differentiation of the limb. Other experiments were, therefore, devised in an attempt to reveal any such effects at this period of development; their aim was to prevent either the formation or any subsequent regeneration of the hind-limb buds.

(2) *Earlier removal of the limb field (Series RME)*

In the next of the series the hind-limb area was removed on both sides at an earlier stage than in the previous experiments. The area of tissue removed was also much greater than before. Larvae which had recently hatched, at stages 35–38 of Nieuwkoop & Faber (1956), were chosen for operation. We removed the whole area of ectoderm and mesoderm covering the posterior half of the endoderm, from the ventral edge of the myotomes almost to the mid-ventral line and extending forwards as far as the fifth or sixth somites. After the operation, the larvae were transferred to Holtfreter's solution at full strength for periods up to 3 days until healing was complete. In the great majority of instances these tadpoles survived the operation, but even here in all but three which grew more slowly than the remainder, hind-limb buds developed from the regenerating body wall.

(3) *Grafting of head epidermis on to the limb area (Series H.Ep.Tr.)*

In this series of experiments, epidermis from the head region was grafted over the prospective limb mesoderm. This skin was grafted at a stage when its presumptive fate was already determined. It has been shown by Balinsky (1931) in *Triton* and *Amblystoma*, and by Filatow (1930, 1932) in *Rana esculenta* that this procedure is capable of suppressing the development of the limbs in these Amphibia.

In our experiments, early larvae of *Xenopus* at stages 35–38 of Nieuwkoop & Faber (1956) were again used. Under narcosis an embryo was stripped of the epidermis covering the prospective hind-limb field; while from another individual was taken the skin of the forehead from the whole area between the eyes and nasal pits. This skin was cleaned from adhering fragments of brain, and transplanted to the hind-limb region of the first tadpole. The graft at first was kept firmly in place by a strip of tantalum foil fixed to the agar floor of the dish. After 1 hr. it was possible to free the tadpole with its adherent graft.

In this series, fourteen larvae received a graft on one side only, while in a further

twelve there was a second transplantation on the other side, two days after the first operation. However, only in a small proportion of instances did these grafts completely suppress the formation of limb buds. Sometimes up to three buds on one side were developed in atypical positions, at the edge of the transplant. The latter usually developed into a large expansion of skin containing nasal pits and filled with a loose mesenchyme within.

(4) *Grafting in parabiosis*

In the next series of experiments, pairs of larvae were grafted together in parabiosis. This method has already been used by Detwiler (1926) to study the effect of peripheral factors on the development of spinal ganglia in *Ambystoma*. He was thereby able to estimate the proportions of extero- and proprioceptive fibres in the sensory nerves of the trunk. In our experiments, the object was to prevent the development of limb buds on the apposed sides of the grafted pairs. Again larvae at stage 33 were chosen for operation. We removed the epidermis of the flank, and thus exposed a large portion of lateral mesoderm and of the myotomes which extended axially from the caudal margin of the pronephros to the hinder limit of the endoderm. Both larvae were then anchored together for 2–3 hr. with their exposed surfaces in contact, at the end of which they were found to be firmly attached. For the next 3 days, they were kept narcotized in half-strength Holtfreter's solution.

Six pairs were grafted successfully, of which only three survived for more than a fortnight; unfortunately they grew only very slowly. One pair kept for 51 days attained a length of 16·3 mm., whereas by then the control animals had reached 39 mm. Study of the sectioned material showed that each pair had a common intestinal tract, which bifurcated anteriorly into the pharynx of each twin. Under these conditions the filter feeding mechanism of the *Xenopus* larvae is in some way rendered inadequate for the nutrition of the grafted pairs.

The primary object of the experiment was however achieved. In the joined larvae only one pair of hind-limb buds were developed, one on either side of the common hind-gut (Pl. 1, fig. 10). Nerve fibres to each hind-limb region came only from the lateral sides of each cord. Yet on the medial side, ganglia S8–S10 were seen to be small-celled. Unfortunately, the development of these paired larvae did not reach the stage when the limb bud begins to influence the growth of the spinal ganglia from which its innervation is derived.

Although the several limitations on these sets of experiments restricted the information which they provided, it can be said that throughout the whole series of experimental larvae, the normal characteristics of their dorsal root ganglia remained unaffected with respect to the relative sizes of their component cells. In all instances where the development of a limb was wholly suppressed either by drastic extirpation of the body wall, or by covering the limb field with head ectoderm or with another larva, spinal ganglia S8 to S10 remained small-celled. In none were there found large neurones similar to the ganglia at non-limb levels. It is thus certain that the character of the lumbar ganglia is intrinsically determined, and in the first place is entirely independent of any stimuli of whatever kind from the periphery.

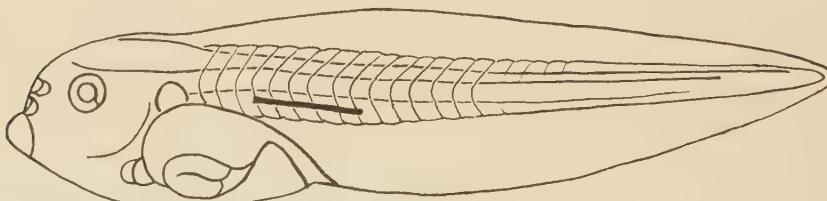
This conclusion is confirmed by results of a fifth series of experiments, different in type from the foregoing, where the aim was not to suppress the development of the limbs, but to hinder their innervation.

This series was free of the limitations which beset the earlier experiments and, moreover, threw some light on the factors which influence the growth in size of dorsal root ganglia. For these reasons this series must now be described in some detail.

Introduction of a barrier between dorsal and ventral regions of the embryo (Series CSL)

The aim of these experiments was to impede the ingrowth of the lumbar nerves into the limb buds, by placing a barrier across their path. A cleft which separated the spinal cord from the hind-limb area was made right through the tail in a frontal plane. The operation was performed on larvae at a length of 8–9 mm. (stage 44–45 of Nieuwkoop & Faber, 1956).

In the narcotized tadpole, a cut, extending from the fifth or sixth somites as far back as the ninth or twelfth was made with a fine steel knife across the central part of the myotomes, and immediately below the notochord, as shown in Text-fig. 4.



Text-fig. 4. Diagram of 9 mm. larva of *Xenopus* showing the initial site and extent of the cleft in the CSL series of experiments.

A thin square of mica was then introduced into the slit. The larvae were returned to tap water immediately afterwards. On recovery they swam about in apparently normal fashion. Within 2 days of the operation, regeneration of the epidermis from its cut edges had covered the mica on both surfaces. After a further 3 days the mica was easily removed; an open and persistent cleft remained.

In the regions of the hind-limb field, these operated tadpoles were divided by a clear space into upper and lower portions. The upper contained the spinal cord, the notochord and most of the somatic musculature; the lower consisted of the abdominal cavity beneath the ventral parts of the myotomes. The dorsal aorta ran through the lower portion, which also included a varying proportion of the hinder part of the mesonephros. From this portion of the body, the hind-limb buds were developed. They appeared towards either the cranial or caudal end of the cleft, for this varied in extent and position from one example to another. Such operated tadpoles lived in an apparently healthy condition for considerable periods; one was not fixed until nearly 2 months after the operation.

The mica was inserted 3–4 days before the hind-limb bud was recognizable on the surface, and about 9 days before any nerve fibres enter the bud. It is thus certain that in their attempt to reach the limb buds the outgrowing pelvic nerves were confronted with the barrier which had been placed across their path. The extent to

which this obstacle was surmounted varied from one individual to another as is shown in Table 2, which summarizes the information derived from the study of seven larvae of this series. The youngest of these is of an age later than that at which nerve fibres normally enter the limb bud.

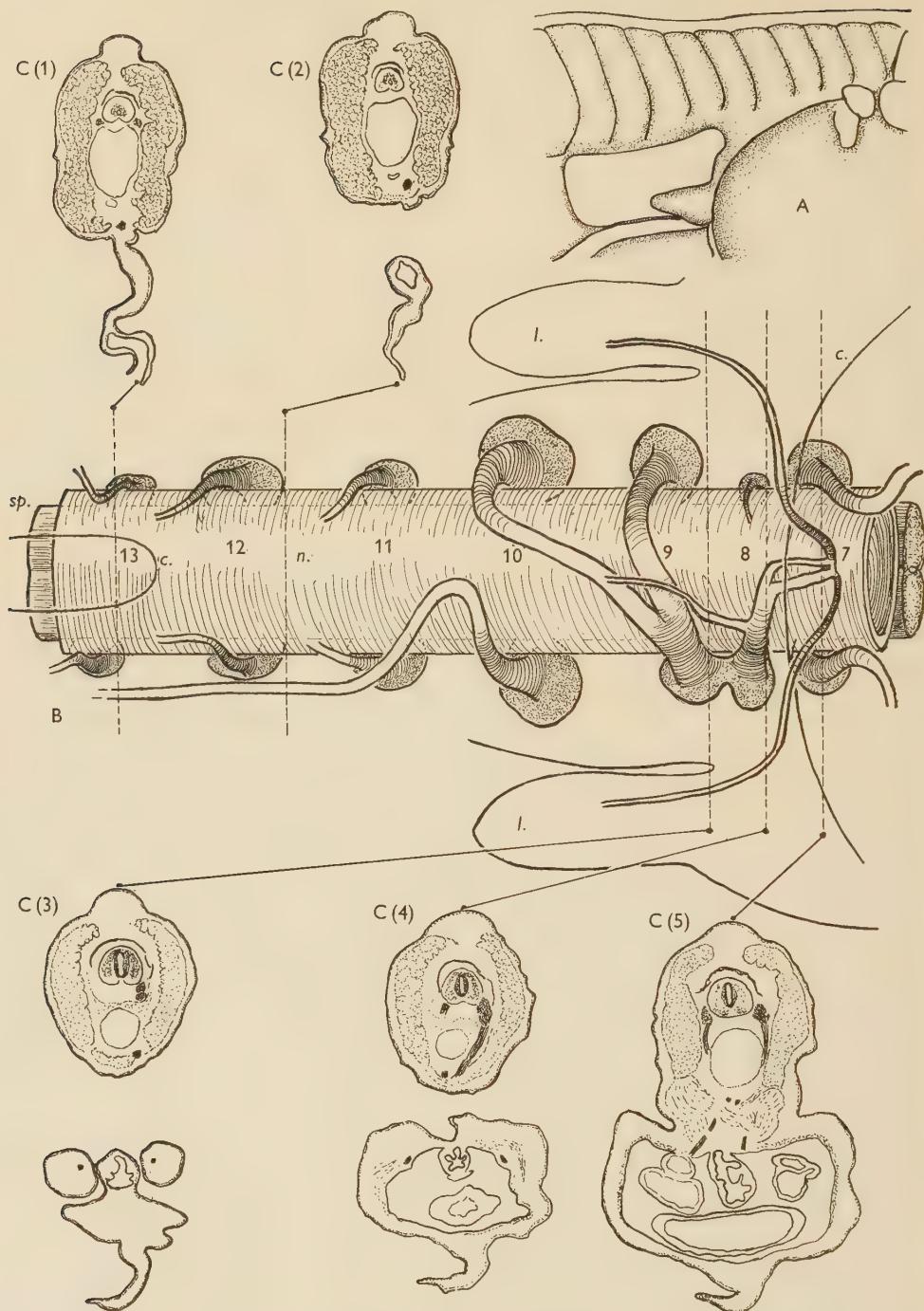
Table 2. Series CSL with cleft in lumbar region

Specimen	Length (mm.)	Days from operation	Extent of cleft in spinal segments	Spinal nerves which reach limb	Relative development of peaks in size of spinal ganglia	Relative development of ventral horns
14.5	18.7	14	8-12	None on either side	Brachial larger	Neither yet developed
17.5	22.0	17	7-9	10 on each side	Lumbar larger	Neither yet developed
21.5	26.0	21	7-9	Right: 8 of both sides+9 Left: 9 only	Lumbar larger	Similar neuroblast stage at both levels
28.5 Text-fig. 5)	30.5	28	8-12	Both limbs: 8 of right side, 9 of both, and 10 of left	Lumbar larger	Brachial more advanced than lumbar
7.6 Text-fig. 6)	33.0	38	8-11	Right: 10 of both sides+11. Left: 11 only	Lumbar larger	Similar at both levels
20.6 Text-fig. 7)	44	51	8-12	Right: None. Left: 7 only	Brachial larger	Right: lumbar absent. Left: small group of cells
25.7	46	86	10-12	Both limbs: 8 with cross-anastomosis	(8 very large, 9 and 10 very small)	Brachial much larger

In the majority of these experimental larvae, some nerves succeeded in reaching the hind-limbs, though in an entirely abnormal arrangement (Text-figs. 5-7). These fibres grew round the artificial gap, and followed its margin through two successive bends at right angles to reach their destination. Of those spinal nerves which thus succeeded in sending fibres to the hind-limb the anterior-most was S7 (Text-fig. 7) which occasionally does so in normal tadpoles. In another tadpole of this series, however, the limb received fibres from the eleventh spinal nerve which normally is outside the limits of the pelvic plexus (Text-fig. 6). Here, however, S12 remained a normal tail ganglion. None of its fibres joined the limb plexus, although their route would have been considerably shorter than that traversed by those fibres from S10 and S11 which succeeded in reaching the limb.

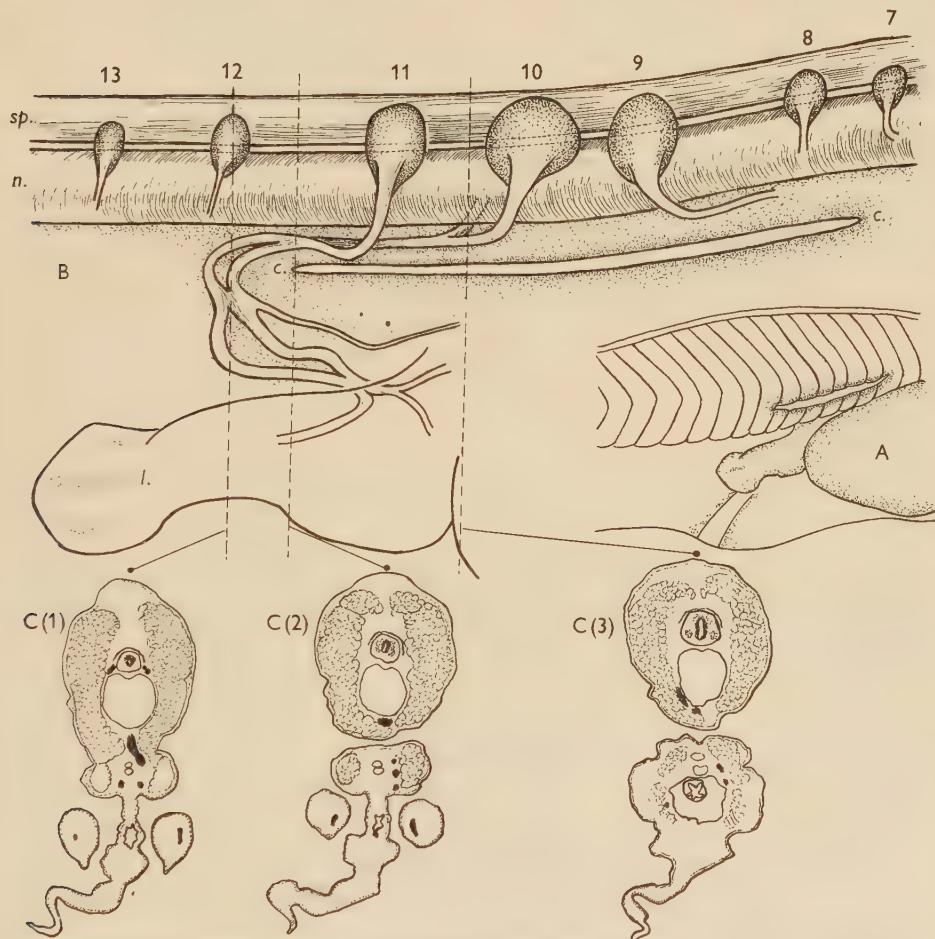
These results suggest that in *Xenopus* the region of the cord competent to innervate a limb is considerably more restricted than in *Ambystoma* (Detwiler, 1936, p. 22). This conclusion is supported by the results of a further series of experiments in which a hind-limb bud was grafted into the tail. Only in one instance, where the limb area was grafted at an early stage, did any fibres from a tail nerve enter a graft. Even here they made little progress and had no perceptible effect on the constituent neurones of the tail ganglion.

A further point which is illustrated by the CSL series is that the limb must extend some attractive influence upon these nerve fibres which are able to respond to the stimulus, of whatever nature it may be. It seems, however, to be effective only over a certain distance; thus in specimen CSL 7.6, S10 grew backwards towards the limb, while S9 made no such attempt and turned forwards to join the sympathetic chain (Text-fig. 6). It is certain that no theory of preformed pathways is relevant to these particular observations. The disturbance of the normal pattern of innervation enhances the interest in the fact that some spinal nerves nevertheless do reach their destination under these unusual conditions.



Text-fig. 5. Specimen CSL 28.5. A: drawn on right side before fixation; B: reconstruction from below in region of cleft. n., notochord; sp., spinal cord; c. limit of cleft; l.l., limb buds; C: (1-5), drawings of sections in planes indicated in (B).

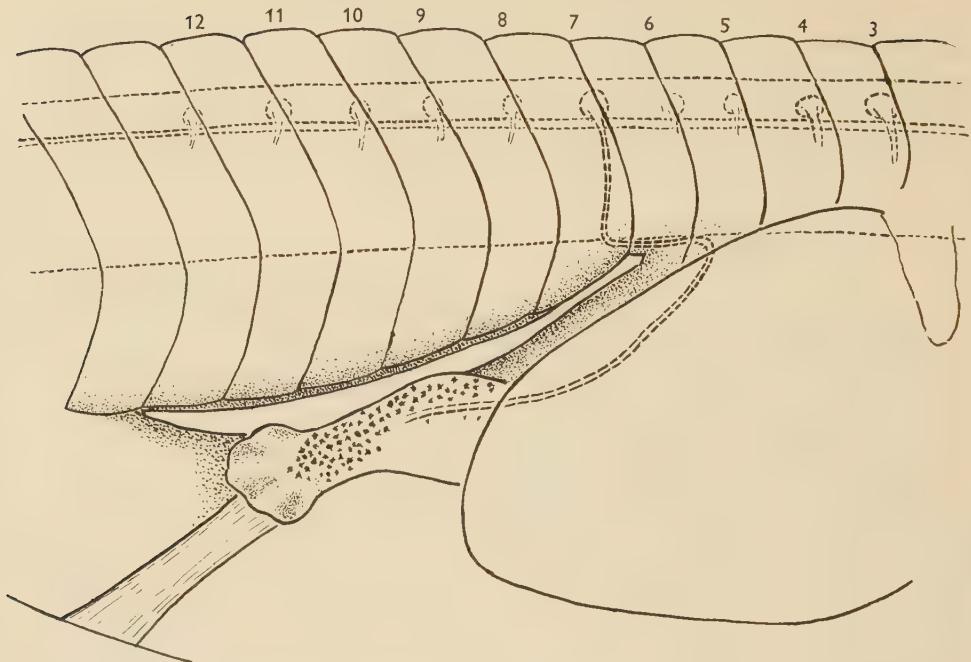
In the normal larva, the nerves to the hind-limb descend from their ganglia and closely follow successively the outlines of the notochord and of the hinder limit of the abdominal cavity. They then swing outwards to enter the limbs (Text-fig. 8). In these experimental tadpoles the spinal nerves also follow the surface of the notochord. In the absence of the further influences which operate in normal development, these nerves often linger near the notochord; they may anastomose over its



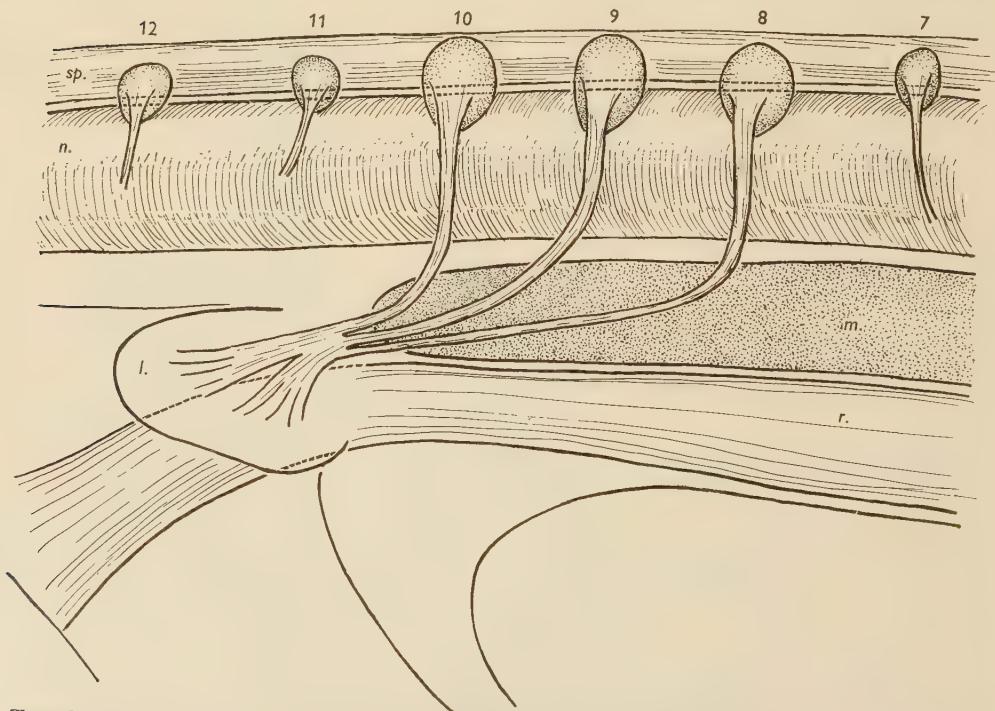
Text-fig. 6. Specimen CSL 7.6. A: drawn on right side before fixation; B: lateral reconstruction from S7 to S13 in region of cleft. n., notochord; sp., spinal cord; c.c., limits of cleft; l., hind-limb.

ventral surface, or they may grow across the median plane. In this way the bilateral symmetry of the whole system of spinal nerves becomes lost. In none of these examples where limbs receive nerve fibres, is the distribution on both sides of the larva the same. There are several instances where nerves cross from one side of the body across to the other beneath the notochord to innervate the opposite limb.

The most striking examples of this are in specimen CSL 28.5 where S9 on one side joins with thick nerves from both S9 and S10 on the other. Fibres possibly from



Text-fig. 7. Specimen CSL 20.6. Drawn on right side before fixation, with course of spinal nerve 7 superposed.



Text-fig. 8. Reconstruction from right side of lumbar region of normal larva at 29 mm. from S7 to S12. n., notochord; sp., spinal cord; m., mesonephros; l., hind-limb; r., rectum.

any of these ganglia then run forwards through the adjacent S8, from which a nerve again follows the notochord round to give off fine branches which loop downwards and finally reach the limb buds (Text-fig. 5).

The composition of the spinal ganglia

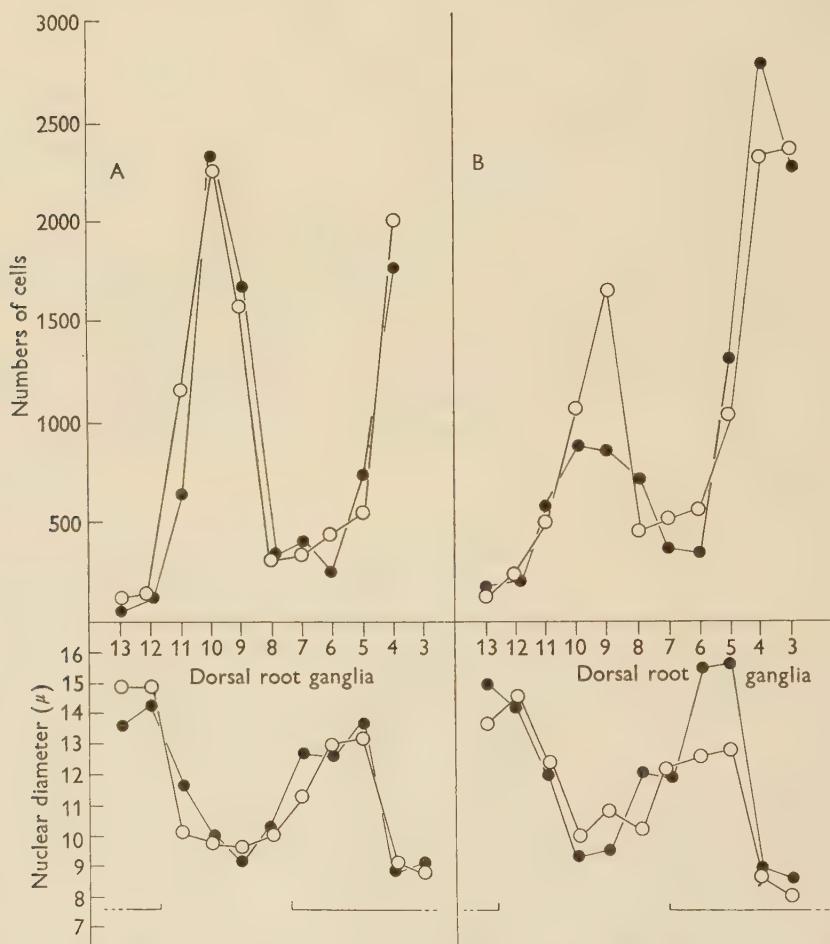
This series of experimental larvae with their variety of pattern of innervation provides further opportunity for the study of the influence of the periphery on the dorsal root ganglia. The whole series as far back as S13 or S14 were accordingly studied in most of these specimens in a similar way to that in which the ganglia of normal larvae have been analysed in the earlier section of this paper (p. 501). We again counted the total number of neurones in each ganglion and measured the nuclear diameter of their largest cells (Text-figs. 2B, 9). The first conclusion which emerges is that throughout the whole series, the normal pattern of relationships remains recognizable. At lumbar levels, both the peak in the number of neurones and also the minimum in nuclear diameter of the largest cells are alike present, irrespective of what nerves the limb receives, or even if none succeeded in reaching it. Specimen CSL 7.6 is the most striking illustration of this generalization, for there the only fibres which enter a hind-limb are a few on one side from S7. It is, therefore, again clear that the normal pattern of the limb ganglia with respect to these features is intrinsically determined.

Imposed upon this self-determined pattern is the effect of the periphery. In terms of the number of constituent neurones within the spinal ganglia, this is expressed in normal development by the two peaks at brachial and lumbar levels. By the time that the pattern of innervation of the limbs is well established the latter peak has become the larger (Text-fig. 2A). In the CSL series, the same is true only where some of the normal lumbar nerves, namely from S8-S10, succeed in reaching the limbs (Text-figs. 2B, 9A). In CSL 20.7, on the side where the hind-limb is without any innervation, the lumbar ganglia are about one-third the size of the brachial (Text-fig. 9B).

There are further differences in detail from one specimen to another of this series. These can most readily be described in comparison with the normal larva, with respect both to cell number and also to the size of the largest nuclei throughout the series of spinal ganglia. In the normal larva, there is a sharp distinction between the 10th and the 11th spinal ganglia. The former is a limb ganglion, the latter typical of the tail. Among the CSL series, however, there are examples where both S10 and S11 belong to either category. In CSL 21.5 the cleft extends caudally only to the level of S9, and here, S10, the fibres of which do not reach the limbs, belongs more to the tail series of ganglia.

On the other hand, where the cleft extends further caudally as in CSL 7.6 and 20.6, even S11 may become similar to a lumbar ganglion (Text-fig. 9). In the first of these two examples, fibres from this ganglion reach the hind-limbs, one of which is wholly innervated from this source (Text-fig. 6). In CSL 20.6, however, the only fibres which reached a hind-limb were a slender bundle on one side from S7; yet here also S11 has developed in the direction of the lumbar type of ganglion. The only abnormal condition which can have influenced its constituent neurones is the effect of the injury at the time of operation and the subsequent regeneration of

ectoderm in an unusual situation. An example from a different experiment may be an illustration of the same type of effect. Among the experimental series RME, where much of the hinder area of the body wall had been removed, the gap was



Text-fig. 9. CSL series. Numbers of cells in spinal ganglia on each side (above) and the diameter of the largest nucleus of each ganglion (below). The longitudinal extent of the cleft is also indicated. A: specimen CSL. 7.6; B: specimen CSL. 20.6, where solid circles refer to the side where the hind-limb received no nerves.

partly closed by apposition of the hind-gut. As a result the eleventh spinal ganglion became small-celled, with a larger number of constituent cells than is usual at this level.

Treatment of the limb area with Nitrogen Mustard (Series LNM)

It has been shown by Tschumi (1954) that if a solution of the Nitrogen Mustard [methyl-bis (β -chloreethyl)-amine hydrochloride] is applied locally to the limb buds of larval *Xenopus*, then the development of limbs may be subsequently inhibited.

Accordingly we adopted this method as a further means of studying the lumbar ganglia in limbless larvae. We soon found that the influence of the Nitrogen Mustard was not confined to the suppression of the legs, but extended to degenerative stages at specific points within the central nervous system itself. These further effects, however, shed further light on the problems in neuro-embryology with which this paper is concerned, though in addition they provide a demonstration of the phenomenon of trans-neuronal degeneration, in which, moreover, the lethal influence is directly manifest as a chain of morbid neuroblasts with pycnotic nuclei.

METHOD

Larvae at stage 4.5 (9–10 mm.), narcotized by the standard procedure, were laid on wet blotting-paper in a Petri dish. The body surface was thus drained of excess water. Small pieces of filter-paper slightly larger in area than the limb field were immersed in solutions of the Nitrogen Mustard at a dilution of either 1:500 or 1:5000. These were then applied to the prospective hind-limb region on one side of the larva for 10 min., during which the dish was kept covered. At the end of this period the tadpoles were returned to water which was strongly aerated. Three series of experiments were made with the higher concentration of the agent. In two of these, each limb bud was treated successively with an interval of 2 days between each application.

RESULTS

At the lower dilution (1:5000) no appreciable effect of the agent was discovered. Over 100 larvae were treated and in none of them did the hind-limbs fail to develop. At the higher concentration, treatment on both sides resulted in the suppression of the hind-limbs in every instance. Here, however, inspection revealed that the influence of the agent was not confined to the extremities. The abdominal cavity became shorter than in control animals of the same age, the ventral fin was partially atrophied, and, in some larvae, the base of the tail was markedly constricted. These general effects on the posterior half of the body were much less marked where the left limb bud alone was treated in each larva, though here the extent of the influence of the substance was very variable (Table 3). In some, the left limb bud was very little smaller than its fellow on the opposite side; in other tadpoles of the series, no limb on the treated side was developed; in a third group, the limb buds on both sides were suppressed, although treatment had been confined to one. This result illustrates how the effect of the substance can spread beyond the area of direct application. Microscopical examination of the section series prepared from these experimental animals also revealed considerable variability in the influence of the agent.

These induced changes call for description under several headings.

A. *Effects within the limb field*

The thickened ectoderm over the early limb bud of the normal tadpoles consists (page 500) of two layers of cells. Within 24 hr. of treatment, the outer ectodermal layer of the limb bud has disappeared, nor does it subsequently re-form. Later, the effect of the substance is seen within the inner zone of the epidermis where the cells enlarge and become irregularly heaped. Individual cells degenerate and the remains

of their pycnotic nuclei are seen within large rounded spaces. In one specimen treated twice with the agent, this change was detected as early as three days after the first application; with one treatment alone, ectodermal degeneration began 4 days later (Pl. 1, fig. 5).

Table 3. *Effects of single treatment with Nitrogen Mustard (series LNM)*

Specimen	Length (mm.)	Days after treatment	Limbs	Spinal ganglia				
				7	8	9	10	11
21.8 A	27.3	19	R.-	+	r	-	-	-
			L.-	+	+	-	-	-
21.8 B	26.4	19	R.+	+	+	+	+	+
			L.-	+	+	-	-	-
21.8 C	27.2	19	R.+	+	+	+	+	+
			L.r	+	r	r	r	+
21.8 D	28.2	19	R.+	+	+	+	+	+
			L.+	+	+	+	+	+
31.8	34	29	R.-	+	r	-	-	-
			L.-	+	r	-	-	-
10.9 A	43	39	R.-	+	r	-	-	r
			L.-	+	-	-	-	r
10.9 B (Text-fig. 13B)	45.5	39	R.+	+	+	+	+	+
			L.-	+	r	r	r	+
15.9 (Text-fig. 13C)	46	44	R.+	+	-	-	+	+
			L.-	r	r	r	r	-

r, reduced.

In an individual fixed 8 days after a single treatment, dividing cells had again appeared within the heaped ectoderm at the sides of the limb bud. Here the mitotic figures were apparently normal and included some late anaphases and telophases. However, in other larvae after two applications of the agent, dividing cells showed the typical effect of 'radiomimetic' poisons on chromosomes and the achromatic figure (Hughes & Fell, 1949). In an example fixed 10 days after the second treatment, irregular groups of chromosomes, some obviously fragmented, were common (Pl. 1, fig. 6). The ectodermal cells were generally hypertrophied, and many were prolonged into blunt pseudopodial extensions. This condition of the ectoderm extended widely beyond the limb area, over the reduced ventral fin and the hinder region of the abdominal surface (Pl. 1, fig. 7). It persists in our material for a further week at the end of which micronuclei, formed by the reconstruction of incomplete groups of chromosomes, are common within an epidermis which consists of several layers of cells.

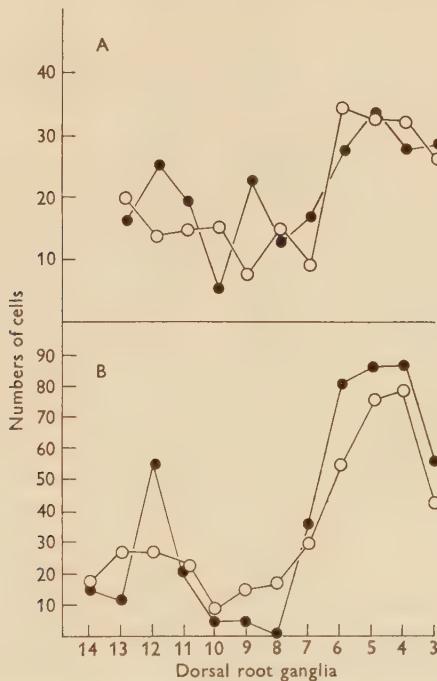
Three weeks after treatment, this appearance of the ectoderm has largely subsided. Outside the limb area its condition approximates to that over the rest of the body surface. Even, however, where no limbs have been developed the sites of the limb buds are marked by an epidermis denser and thicker than elsewhere which consists of several layers of cells with rounded nuclei, among which degenerations still continue (Pl. 1, fig. 8).

Changes within the mesodermal cone of the limb bud are much less spectacular. This component of the limb bud is steadily reduced in volume as the changes within the ectoderm proceed, though usually with rather fewer pycnotic cells in evidence.

It seems that the injured ectoderm can no longer evoke the production of cells within the mesodermal core. Persistence of this condition results in the suppression of the limb.

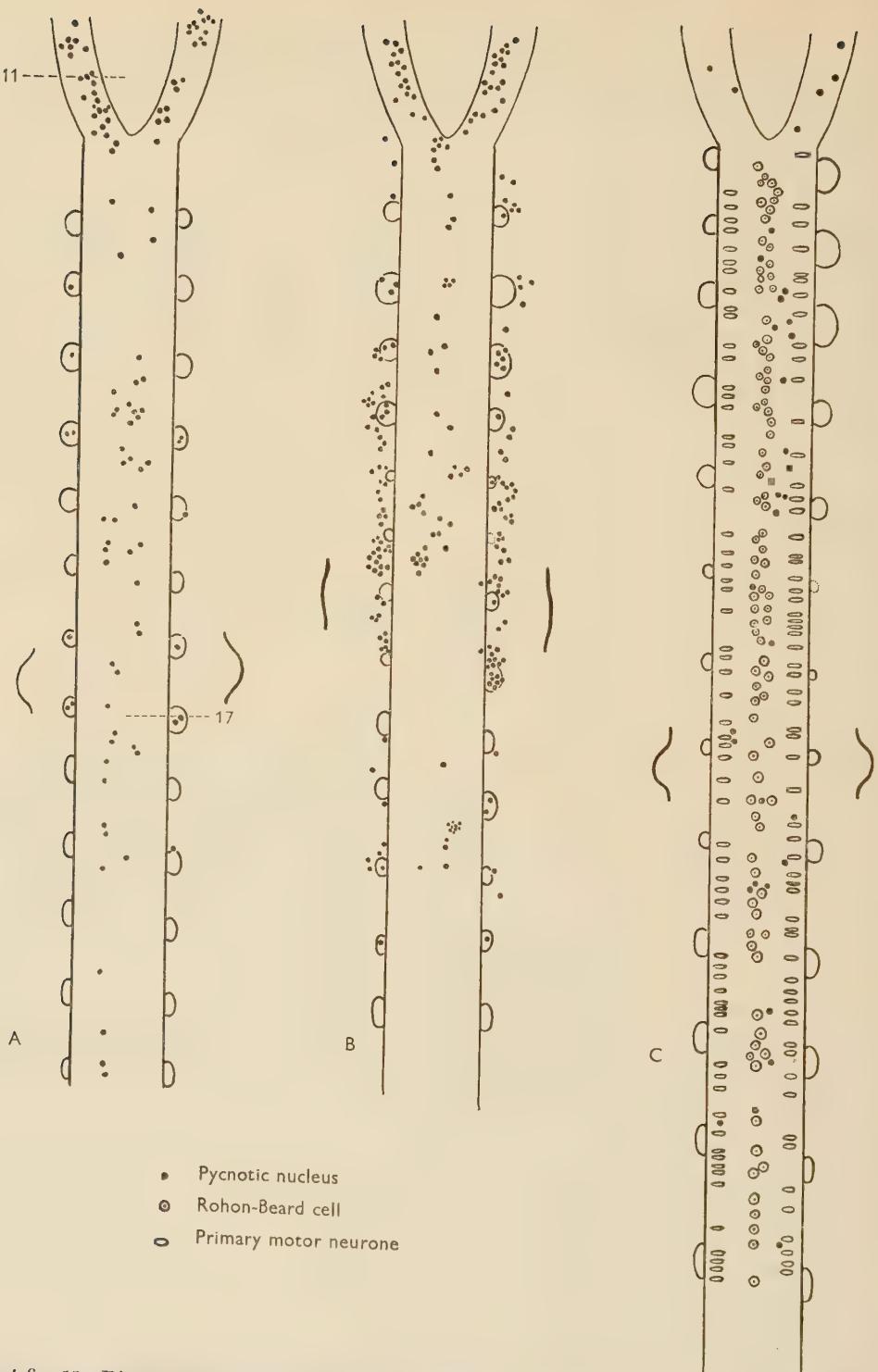
B. Degeneration within the dorsal root ganglia

The application of the Nitrogen Mustard to the limb area had the further consequence that neuroblasts degenerated within the dorsal root ganglia. The earliest stage at which pycnotic nuclei at these sites were seen was in a tadpole fixed four days after a single treatment (Pl. 2, fig. 17). This secondary effect of the agent must follow soon after degeneration has begun within the inner layer of the epidermis of the limb area. The cells within each spinal ganglion were counted (Text-fig. 10A)



Text-fig. 10. Numbers of cells in spinal ganglia on each side in two tadpoles fixed at different times after a single application of Nitrogen Mustard. A: 10.9 mm., 4 days after treatment; B: 14.2 mm., 8 days after treatment.

and can be compared with those of the normal larvae at approximately the same stage (Text-fig. 1A). It is thus seen that those ganglia which would normally innervate the whole limb field, namely S7 to S11 on each side, have already lost about twenty cells each. The frequency of pycnotic nuclei within them does not by itself suggest the extent to which cells had already been lost (Text-fig. 11A). Eight days after a single treatment (Text-fig. 11C) pycnotic nuclei within the dorsal root ganglia are no longer found, but counts of the remaining cells therein show that on both sides ganglia S7-S11 have lost the majority of their cells and from S8-S10 on the treated side very few neuroblasts now remain (Text-fig. 10B).



Text-fig. 11. Diagrammatic reconstructions of spinal cord and ganglia in three tadpoles treated with Nitrogen Mustard, to show pycnotic nuclei. A: 10.9 mm. 4 days after single treatment (cf. Text-fig. 10A), the levels indicated are of figures 11 and 17 in Pls. 1 and 2; B: 14 mm. 6 days after second treatment; C: 14.2 mm. 8 days after single treatment (cf. Text-fig. 10B). Rohon-Beard cells and primary motor neurones shown in addition to pycnotic nuclei.

Where the agent was applied twice, cell degeneration around the cord continues for a more extended period. In a specimen fixed 6 days after a second treatment, and thus comparable to the tadpole of Text-fig. 11C, pycnotic nuclei are frequent at levels between S6 and S10, though their distribution extends beyond the neuroblasts of the dorsal root ganglia to the undifferentiated cells between (Text-fig. 11B).

In larvae fixed several weeks after treatment with Nitrogen Mustard, and where dorsal root ganglia are missing, masses of cells are sometimes seen around the corresponding ventral roots of the cord. These aggregations consist of macrophages, probably derived from the Schwann cells of the ganglia (Weiss, 1944), together with fibroblastic cells and engorged blood vessels. Such an appearance suggests a removal of cell debris, and may imply that neuroblasts of the spinal ganglia have only recently degenerated.

C. Degeneration within the cord and hind-brain

A further effect of treatment with the Nitrogen Mustard is a general destruction of tissues at the tip of the tail. This spreads into the caudal tip of the spinal cord, and provokes a continuous tract of cell degeneration in the mid-dorsal sector, which extends forwards for a few segments. Through the remainder of the cord, pycnotic nuclei are less abundant, and are scattered through its dorsal half. In the hind-brain, there is again a more concentrated tract of degeneration on either side of the fourth ventricle (Pl. 1, fig. 11). This degeneration within the cord and medulla has already begun in the larva fixed 4 days after a single treatment (Text-fig. 11A). After a further 4 days, the number of pycnotic nuclei is somewhat lessened (Text-fig. 11C).

In this figure, both the Rohon-Beard cells and the primary motor neurones of the cord are also represented. There is no indication that the number of these two types of differentiated neurone has been decreased by the effects of the Nitrogen Mustard.

The pycnotic nuclei in the hind-brain are confined to the mantle layer, and do not affect the germinative zone adjacent to the ventricle. In this region, normal mitotic figures continue throughout the period when mantle neuroblasts are degenerating. This fact eliminates the possibility of a general effect on the whole medulla oblongata of the Nitrogen Mustard, for dividing cells would be expected to be more susceptible than post-mitotic neuroblasts to its action.

Some 10 days after treatment, mitotic figures are commoner in the hind-brain and in the cord at trunk levels than is so in control tadpoles of the same age. This compensatory cell-division is, however, apparently insufficient to restore the population of cells within the cord to its normal level. In the tail the transverse sections of the cord often become irregularly fenestrated, particularly in the dorsal half where in places only processes of the sustentacular cells remain. At trunk levels the central canal becomes dilated.

D. Propagation of degenerative changes

The mitotic figures within the cord and the hind-brain are all normal in character, with the expected proportions of anaphases and telephases. This fact suggests that the Nitrogen Mustard did not of itself penetrate to these levels, but that here, cell degeneration was due to a secondary lethal influence transmitted in some way along the ascending tract of the cord, and without effect on the dorsal sensory neurones

within the cord, the Rohon-Beard cells. Where the Nitrogen Mustard had been directly in contact with tissues of the larva, permanent genetic effects on cells are evoked. The abnormal mitotic figures in the epidermis of the limb area have already been mentioned (p. 515 above); again, within the hind-gut epithelium of larvae which received a double treatment, to which the substance may be assumed to have reached by direct diffusion, micronuclei were seen a fortnight after the second application of the agent. At later stages in this group of experimental tadpoles, the thick epithelium of the hind-gut shows conspicuous areas with giant nuclei (Bodenstein, 1947). Such zones are sharply demarcated from neighbouring regions where the nuclei are of normal size (Pl. 1, fig. 9).

At the surface of the cord, it is necessary to distinguish between two types of effect of the agent. After the double treatment, frequent pyknotic nuclei were here seen (Text-fig. 11 B) not confined to the lumbar region of the cord nor limited to the dorsal root ganglia themselves. Some bizarre consequences of the destruction of cells in this region were subsequently seen. In a larva fixed 19 days after the first application of the agent, there are large neurones within the myotomes opposite dorsal root ganglia S₅ to S₇. In another, fixed 17 days later, a sheath of striped muscle fibres closely enveloping the dorsal and lateral surfaces of the cord in the lumbar region had been formed. In this tadpole, alone of all those treated with Nitrogen Mustard, the arrangement of the ventral roots of the cord was disturbed. In the lumbar region of this specimen, numerous adventitious rootlets arose from the lateral surface of the cord in no orderly segmental arrangement. Such effects are probably the consequences of the diffusion of the agent, or of some secondary product through the tissues of the larvae to the surface of the spinal cord.

On the other hand, in the series of larvae which received only a single treatment with the agent, abnormality at the surface of the cord was confined to the loss of dorsal root ganglia and to an extent which varies from one specimen to another (Table 3). Where a ganglion was entirely absent its level was indicated by the corresponding motor root on the ventral surface of the cord (Pl. 2, fig. 18) and the intervertebral foramen through which these fibres emerge. Table 3 shows that the loss or reduction of dorsal root ganglia is confined to S₇ to S₁₁ inclusively, i.e. those which usually innervate the limb, together with S₇ and S₁₁ which belong to the periphery of the limb field and are capable of sending fibres to the leg as an abnormality. The ganglia of S₈ to S₁₀ are, however, those most frequently lost or reduced.

The substance was applied at a stage a few days before nerve fibres entered the limb buds. Within a day of treatment some lethal influence from the degenerating cells of the limb area must reach the growing lumbar nerve fibres in the region of the somatopleuric band of muscle. Dorsal root fibres are then selectively destroyed; motor fibres can be seen to persist in this region after treatment, and the ventral roots of the spinal nerves remain unaffected. We must, therefore, assume either that motor fibres are more resistant than sensory to a lethal influence which spreads inwards from the limb bud or that dorsal root fibres are capable of transmitting the effect in an afferent direction.

The ventral horn

At stages 50–51 of Nieuwkoop & Faber (1956) a compact rounded group of neuroblasts is formed at the ventro-lateral border of the grey matter of the cord at brachial and lumbar levels on each side. The former is concentrated at the level of S4, while the latter in normal larvae is first found between S8 and S9 (Pl. 2, fig. 13). Later the lumbar horn extends caudally to the level S10 and rostrally to a variable point between S7 and S8.

By stage 52, neuroblasts of the ventral horn begin to differentiate into bipolar neurones. The first to mature are a lateral group. The nuclear membrane and the nucleolus enlarge; the general background of the nucleus stains less densely. The cytoplasm becomes distinct, as the first Nissl material is deposited therein. At stage 54, a maximum of about twenty such neurones on each side is seen at the level of S9. Not all neuroblasts differentiate into neurones. During the second stage of its development, pycnotic nuclei are seen among the cells of the ventral horn. As the neurones increase in size, they are reduced in number (Nieuwkoop & Faber, 1956, p. 70).

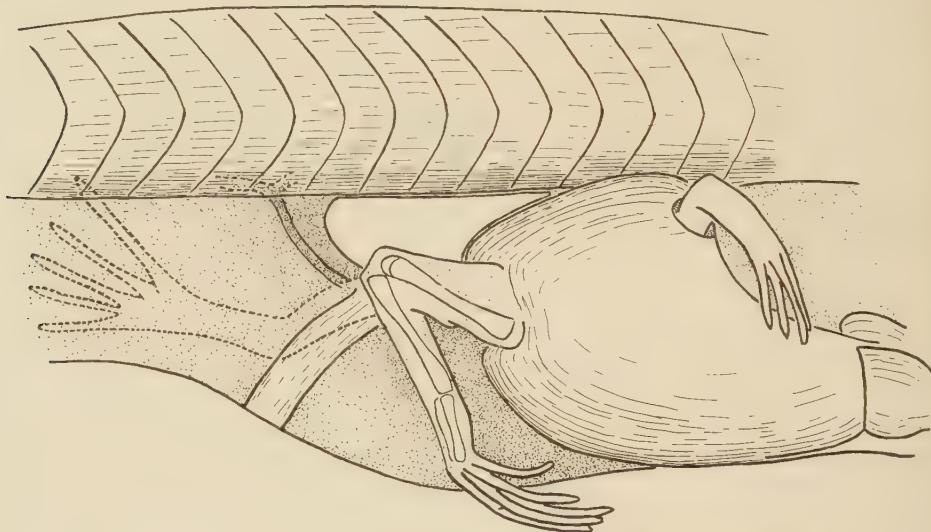
The neuroblast stage in the development of the lumbar horn is reached while the hind-limb buds are still small. Examples of larvae from the various experimental series show that the absence of a limb bud on one side does not inhibit the first stages of development of the ventral horn (Pl. 2, figs. 15, 16). In particular, a specimen (RME 15.9A) where the body-wall remained incomplete on one side over an area which was closed by the hind-gut wall, has a ventral horn on both sides which extends caudally to an unusual extent; here one effect of the extensive injury was to cause the ganglion of S11 to develop as a member of the lumbar series. Again, in CSL 7–6, where ganglion S11 is also of this type, the ventral horn reaches nearly to this level. It seems, however, that while the extent of the longitudinal development of the ventral horn up to this stage is influenced by character of the dorsal root ganglia, some neuroblasts will be formed even in the absence of the ganglia. This point is demonstrated by specimen LNM 28.5, where a double treatment with Nitrogen Mustard resulted in the suppression of both limbs and all limb ganglia. Yet ventral horn neuroblasts are here present within the cord.

The necessary conditions for the differentiation of neuroblasts of the ventral horn into mature neurones are, however, more exacting. One of these is contact with the periphery by a normal lumbar nerve. In specimen (CSL 20.6), the general development of which corresponds to stage 54, no lumbar nerve reached the left limb; on this side within the cord no ventral horn is to be found (Pl. 2, fig. 19), and the original neuroblasts must have been dispersed within the mantle layer of the cord. On the right, some fibres from S7 succeeded in reaching the limb. A few scattered groups of neuroblasts between the levels of S9 and S10 represent the ventral horn on this side. Although the lumbar spinal ganglia even on the left side consist of about 800 cells each, this by itself is not sufficient to permit a differentiated ventral horn to be formed.

In specimen CSL 25.7, S8 on each side reached the limb. At this level the dorsal root ganglia are very large indeed, yet the ventral horn consists only of a small group of neurones in its immediate neighbourhood (Pl. 2, fig. 21). These were not adequate

to evoke the normal function of the hind-limbs. Both legs were paralysed, and the only movement seen in them was slight tremor of the third and fourth digits on one side (Text-fig. 12).

A second condition for the development of ventral horn cells in normal numbers is the presence of a minimal proportion of cells within the dorsal root ganglia. The later members of the series which received one treatment with Nitrogen Mustard are



Text-fig. 12. Specimen CSL 25.7 drawn before fixation. Both limbs were paralysed, and were innervated only by S8.

at stages when differentiated motor neurones are normally present. In these tadpoles, the ventral horn is reduced by a varying amount which depends upon the extent to which the dorsal root ganglia are lost (Pl. 2, fig. 20). The most extreme degree of reduction is seen in specimen LNM 10.9A, where there remain only a few cells of ganglion S8 on the right side; here, the ventral horn is confined to a small group of differentiating motor cells in the neighbourhood of this ganglion. As in the rest of the series, the ventral roots are all present in this specimen. This circumstance suggests that the stimulus from the limb which leads to the differentiation of a ventral horn is transmitted along afferent fibres.

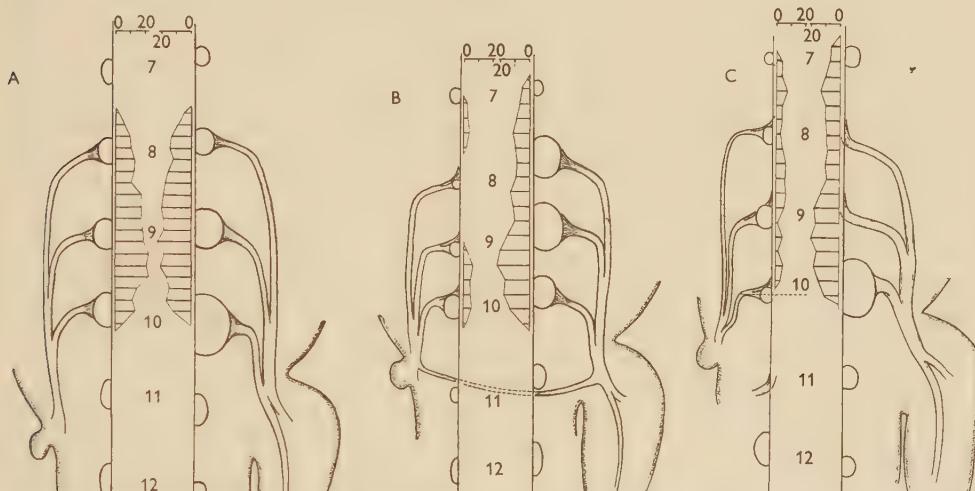
In specimen LNM 15.9 where the hind-limb persisted on the right side, the dorsal root ganglia of S8 and S9 here were lost, while in compensation, S10 developed into a huge ganglion. The ventral horn on this side is best developed at this level, but it extends forwards on a reduced scale as far as S7 (Text-fig. 13C).

In both specimens LNM 10.9B (Text-fig. 13B) and LNM 15.9, a small limb bud succeeded in regenerating on the left side. The development of the ventral horn in these tadpoles can be compared with that in one of the same stage, where the same degree of limb reduction was achieved by simple ablation (specimen HLRF, 27 days: Text-fig. 13A). In the latter, the ventral horn on the operated side is almost normal in size.

There are two possible explanations of the difference between these examples from the two series of experiments:

(1) The Nitrogen Mustard was applied before a limb bud was present, whereas the ablation experiment was performed after the bud had appeared.

(2) The reduced ventral horn on the left side in these examples from the LNM series may be a response to a stimulus from the regenerating limb bud, restricted in its inflow to the cord by the loss of dorsal root neurones.



Text-fig. 13. Diagrammatic reconstructions of spinal cord and ganglia in the lumbar region, together with graphical representations of the ventral horns, in which the average number of differentiated neurones in three consecutive sections is plotted on scale shown at ten section intervals. In all three, the left limb is greatly reduced. A: 44.5 mm., left limb ablated 27 days before fixation, ventral horn on this side nearly normal; B: specimen LNM 10.9 B, 45.5 mm., fixed 39 days after single treatment with Nitrogen Mustard, a cross-anastomosis connects the lumbar plexus on each side; C: specimen LNM 15.9, 46 mm., fixed 44 days after single treatment with Nitrogen Mustard (dotted line shows level of section in Pl. 2, fig. 20).

These explanations are not incompatible; to decide between them it would be necessary to know for how long during development the peripheral stimulus for the differentiation of the ventral horn remains effective.

DISCUSSION

Two main points emerge in the foregoing pages. The existence of self-differentiating factors in the dorsal root ganglia is the first of these, and has already been discussed. The second concerns the development of the ventral horn and here the present findings need to be considered in relation to those of other workers. The dependence of the ventral horn on peripheral factors has already been demonstrated by a number of authors. Among these, Dürken (1911), Hamburger & Keefe (1944) and Barron (1945), working respectively with *Rana*, the chick and the sheep foetus, have shown that ablation of a limb results in the hypoplasia of the ventral horn cells within the cord. The same conclusion has been reached by grafting a second spinal cord alongside the host cord (May, 1930; Bueker, 1943).

Here our main concern is with the views of certain of these authors on the means through which the influence of the periphery is exerted during development.

Barron (1943) suggested that if, as Ramon y Cajal believed, motor neuroblasts form their dendrites only when the axon has reached the periphery, these dendrites might serve to induce neighbouring neuroblasts to differentiate further. In their turn the latter would develop both axons and dendrites. So a wave of differentiation of cell-bodies within the cord would keep pace with the increase in the number of axons arriving at the growing limb. This might continue until all the developing muscle fibres within the limb had received their innervation.

This hypothesis was slightly elaborated by Hamburger & Keefe (1944) who point out, however, that hypoplasia of the ventral horn can be already demonstrated in a 5-day chick when a limb has been removed at 3 days of incubation. Peripheral control is thus effective long before the myoblasts of the limb have differentiated very far.

That a stimulus from the periphery is necessary for the development of the ventral horn in *Xenopus* is shown in the present work by the CSL series of experiments. Where a limb received no innervation no motor neurones were differentiated within the corresponding region of the cord. However, where lumbar nerves are present a limb bud in an early stage of development is sufficient to evoke the formation of a ventral horn nearly of normal dimensions.

In 1943, Barron showed that if the forelimb of the sheep foetus was severed at 60 days of foetal life, then a hypoplasia on one side of the whole cord in the brachial region can be seen towards the end of normal gestation. He regarded the observed decrease in volume of the *substancia gelatinosa* and of the intermediate grey matter within the cord as a consequence of the reduction in size of the dorsal root ganglia in the first place. The decrease in volume of the ventral horn, however, which was the most marked of all these hypoplastic changes, he thought was due mainly to a retrograde degeneration of neurones which were present at the time of the amputation, and thus 'not of assistance in the analysis of the problem at hand'.

In *Xenopus*, however, the primary stimulus from the periphery which results in the development of the ventral horn, appears to be transmitted through dorsal roots, though the present evidence does not eliminate the possibility that the axons of ventral roots may act in the manner which has been suggested by Barron.*

Recently, there has been particular emphasis on the role of the periphery in maintaining central neurones through functional stimulation. Where nerve cells do not receive or are deprived of such stimuli they degenerate (Hess, 1957).

In comparing the observations of workers on the amniote ventral horn with those here described, it must be borne in mind that the neuroblast stage in the differentiation of a nerve cell is not necessarily equivalent in all vertebrates (Hess, 1957). In *Xenopus*, the motor horn cells are bipolar throughout their development, whereas in the chick (Barron, 1946) they reach this condition by the loss of all dendrites but one. Again, the deposition of cytoplasmic ribonucleic acid within these cells follows a different course in the two groups: in the chick there are distinct peaks in

* Possibly there is evidence of this from specimen LNM 15.9 (Text-fig. 13C) where a reduced ventral horn extends forwards through S8 and S9, the dorsal root ganglia of which have disappeared. However, these ganglia may have degenerated at a relatively late stage.

concentration which may be related respectively to differentiation and function (Hughes, 1955), while in *Xenopus* the density of this substance remains high throughout the stages of the neuroblast and the differentiated neurone.

SUMMARY

1. The development of the dorsal root ganglia and ventral horn in the lumbar region of the larva of *Xenopus laevis* has been studied, both under normal and experimental conditions, with the object of determining the influence of the hind-limb on these nervous units.

2. At mid-larval stages, limb ganglia differ from those at non-limb levels in two respects. The former are made up of a great number of cells, while the latter contain some cell bodies much larger than are found in the limb ganglia. These differences between the two types of ganglia can be recognized before the entry of nerve fibres into the limb.

3. The cord and ganglia have been freed from the influence of the developing leg in several ways: by surgical ablation of the limb, by its destruction with Nitrogen Mustard, and by interposing a barrier between the outgrowing spinal nerves and the periphery.

4. The normal increase in the number of cells within the lumbar spinal ganglia is retarded when deprived of the influence of the limb. The size of their constituent neurones is not affected thereby.

5. The ventral horn develops in two phases: first, a neuroblast stage which is independent of peripheral influence, and secondly the stage of differentiated neurones. This stage is attained only when a lumbar nerve has reached the limb. A ventral horn of nearly normal size is formed when a limb is ablated at an early stage.

6. When the limb area is treated with Nitrogen Mustard, the development of the leg may be suppressed. The degenerative influence spreads to the fibres and cells of the lumbar spinal ganglia, but leaves ventral roots unaffected. Reduction of both limb and lumbar ganglia in this way results also in severe depletion of neurones in the ventral horn.

7. It seems that the early limb-bud transmits some trophic stimulus mainly along afferent fibres to the dorsal root ganglia, and through them to the developing ventral horn, causing neuroblasts to differentiate into neurones.

We wish to thank Prof. J. D. Boyd and Dr Gordon Wright for helpful discussions during the course of this work, and Mrs J. Branch for her invaluable technical assistance. The expenses of this research were borne by a grant from the Nuffield Foundation to the School of Anatomy.

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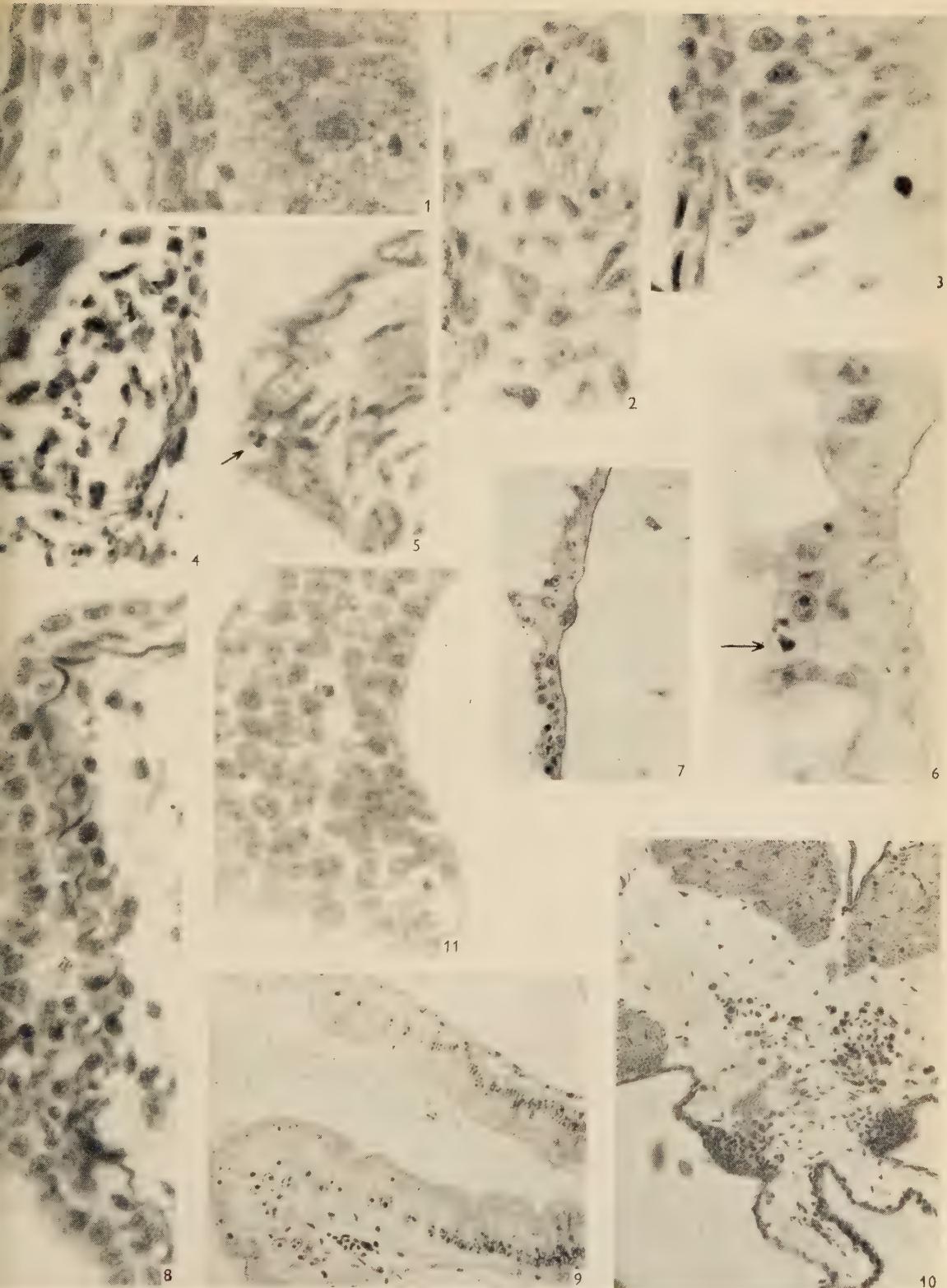
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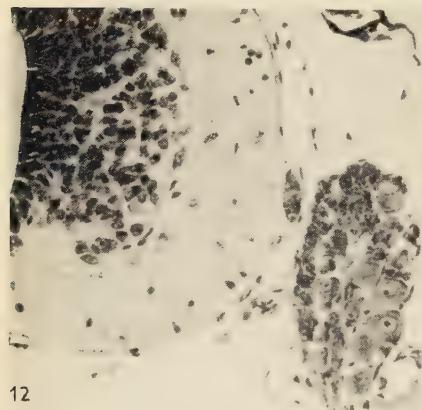
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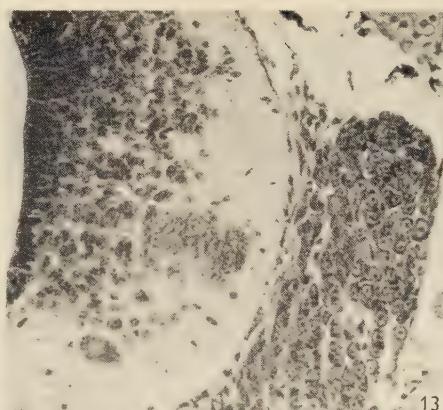
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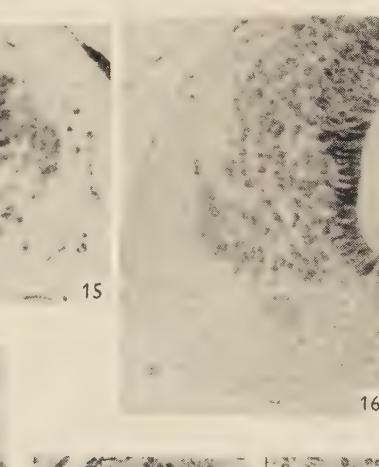


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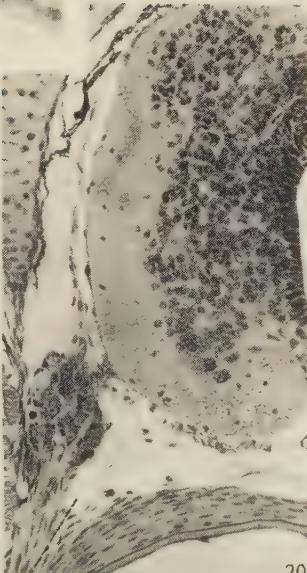
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Transverse section through presumptive hind-limb area of 5.7 mm. tadpole, from ectoderm (left) to gut endoderm (right). Mesoderm surrounding gut as yet undifferentiated. $\times 610$.

Fig. 2. Transverse section through hind-limb area at 6.0 mm. stage, showing the group of myoblasts (upper half) which will differentiate into the somatopleuric muscle band. Ectoderm to left. $\times 610$.

Fig. 3. Transverse section through hind-limb area at 9.0 mm. stage. Ectoderm (left) is two-layered; to the right is the mesodermal cone. $\times 610$.

Fig. 4. Transverse section of base of limb bud at 18 mm. stage, showing entry of first nerve fibres (lower third). Top left: somatopleuric muscle band; on right, coelomic wall, in contact with which nerve fibres approach limb area. $\times 610$.

Fig. 5. Transverse section through limb bud of 10.9 mm. tadpole, 4 days after single treatment with Nitrogen Mustard, showing pycnotic nuclei in ectoderm. $\times 610$.

Fig. 6. Transverse section through part of limb area of 17.2 mm. tadpole, 10 days after second treatment with Nitrogen Mustard, showing pseudopodial extensions of ectoderm cells, and in one cell, an irregular group of chromosomes. $\times 610$.

Fig. 7. Transverse section through part of ventral fin of 18.5 mm. tadpole, 13 days after second treatment with Nitrogen Mustard, showing irregular condition of ectodermal surface. $\times 180$.

Fig. 8. Transverse section through limb area of 27.3 mm. tadpole, 19 days after single treatment with Nitrogen Mustard, showing thickened ectoderm with one pycnotic nucleus (just below centre of picture). $\times 610$.

Fig. 9. Part of transverse section through hind-gut of 26 mm. tadpole, 34 days after second treatment with Nitrogen Mustard, showing diversity in size of gut cells. $\times 180$.

Fig. 10. Part of transverse section through common hind-limb buds of parabiotic twins, 16.3 mm. long. The dorsal region of each twin lies respectively to left and right. Opening of common hind-gut on lower right of picture. $\times 125$.

Fig. 11. Transverse section through half of hind brain of 10.9 mm. tadpole, 4 days after single treatment with Nitrogen Mustard, showing three pycnotic nuclei among the medullary neuroblasts. $\times 610$. The level is indicated in Text-fig. 11A.

PLATE 2

Figs. 12–14. Transverse sections through halves of spinal cord and dorsal root ganglia of 35 mm. tadpole at three levels. $\times 170$. Fig. 12. Through ganglion 7 (with large neurones). Fig. 13. Through ganglion 10 (ventral horn in neuroblast stage, limb ganglion with small neurones). Fig. 14. Through ganglion 11 (with large neurones).

Fig. 15. Transverse section through half of spinal cord in lumbar region of specimen RME 15.8 A, 22.8 mm. long, showing ventral horn in neuroblast stage developed in the absence of a limb. $\times 170$.

Fig. 16. Transverse section through half of spinal cord in lumbar region of specimen H.Ep.Tr. 20.10, 42.5 mm. long, again with ventral horn in neuroblast stage, developed in the absence of a limb. $\times 170$.

Fig. 17. Transverse section through half of spinal cord and spinal ganglion 10 of 10.9 mm. tadpole, 4 days after single treatment with Nitrogen Mustard, showing a degenerating nucleus within the ganglion. $\times 610$. Level of section shown in Text-fig. 11.

Fig. 18. Transverse section of half of spinal cord of specimen LNM 31.8, 34 mm. long, 29 days after single treatment with Nitrogen Mustard, showing ventral root of S9, the dorsal root ganglion having been destroyed. $\times 125$. (Dotted line between nerve and fibrous sheath of notochord.)

Fig. 19. Transverse section through half of spinal cord of specimen CSL 20.6, 44 mm. long at level of spinal ganglion 9. $\times 125$. No nerve reached the leg, and there are no ventral horn neurones in the cord.

Fig. 20. Section through left half of spinal cord of specimen LNM 15.9 A, 44 mm. long, 44 days after single treatment with Nitrogen Mustard. The section is at the level of the 10th spinal ganglion, which on this side was greatly reduced (Text-fig. 13C). No ventral horn neurones here have differentiated. $\times 125$. (Dotted line between nerve and fibrous sheath of notochord.)

Fig. 21. Transverse section through right half of spinal cord of specimen CSL 25.7, near the eighth spinal nerve, which alone reached the limb. A few differentiated neurones in the ventral horn are seen, one pycnotic nucleus is among them. $\times 125$.

THE CAPSULE OF SPINAL GANGLION CELLS

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There are various accounts of the nature of the capsular elements of spinal ganglion cells. De Castro (1931) describes a 'corona' of spindle-shaped satellite cells with branching processes within an external covering of collagenous and reticular fibres. The intracapsular part of the axon, he states, is tortuous and convoluted forming a 'glomerulus'. Ranson's *Anatomy of the Nervous System* (1953) shows a nucleated membranous sheath continuous with the axon neurilemma and satellite cells embedded in the substance of the nerve cell, while Cajal's pictures, reproduced in Maximow & Bloom's text-book (1957) have a discontinuous layer of satellite cells, some of them enclosed by dendritic loops arising from the surface of the nerve cell, separated by a clear space from the cell body. In haemalum- and eosin-stained sections examined with the light microscope there is a 'shrinkage?' space around most of the ganglion cells, limited externally by a capsule of cells amongst which are fibroblasts, while in a number of neurons nuclei indent the cell surface.

In previous reports on the fine structure of spinal ganglion cells (Hossack & Wyburn, 1954; Dawson, Hossack & Wyburn, 1955; Dawson & Wyburn, 1955) mention was made of the capsular elements, but with the thick sections produced at that time by a modified Spenser microtome no detailed interpretation was attempted.

MATERIAL AND METHODS

The spinal ganglia of normal adult rabbits were used. The animals were anaesthetized with Nembutal and the ganglia removed before death, placed in a few drops of fixative on a glass slide, cut into one or two pieces with a sharp razor blade, and quickly transferred to a dark tube containing Zetterquist (1956) 1% buffered osmic acid.

The fixation was continued for 4 hr. at room temperature, the tissues were washed overnight, then were dehydrated through a graded series of alcohols. One hour in each change was sufficient to ensure preliminary dehydration; final dehydration was effected by a 2 hr. immersion in alcohol which had been re-distilled over calcium hydride.

Following several changes of *n*-butyl methacrylate the tissue was embedded in a mixture of pure *n*-butyl methacrylate containing 5% methyl methacrylate, and 1% benzoyl peroxide as catalyst, before being finally embedded in gelatin capsules. Polymerization was carried out by placing the capsules in an oven at 56° C. for 3–6 hr.

Sections of from 500 to 300 Å. were cut with a Cooke and Perkins microtome, mounted on copper mesh grids, and examined in the Philips E.M. 100.

DESCRIPTION

Each ganglion cell is closely and completely invested by a sheath of nucleated cytoplasm, including the area of the 'implantation cone' of the axon. The narrowest part of the sheath—between the nuclei—is $\pm 0.5\mu$; and the widest—at the nuclei—is $\pm 3\mu$ (Pl. 1, figs. 1, 2). Only the broader regions, therefore, can normally be seen with the light microscope giving the impression of nuclei indenting the nerve cell surface at intervals. The satellite nuclei more often than not occur in pairs separated by the cell membranes, so that the broad ends of adjacent cells are in contact (Pl. 1, fig. 1). It is more difficult to see the cell boundaries in the inter-nuclear regions (Pl. 2, fig. 4). The satellite sheath thus appears in section as pear-shaped cells with long tapering processes, so arranged that the broad ends and the narrow ends of the cells are adjacent to one another. The satellite cytoplasm tends to be lighter in texture than the nerve cell cytoplasm, largely due to the absence of Nissl's substance, although there are scattered accumulations of small granules very similar to those of the Nissl aggregations, and the mitochondria, like those of the nerve cell, are small, dense and spherical. No endoplasmic reticulum or Golgi substance was seen in the sheath cytoplasm. The satellite sheath is bounded externally by double membranes seen in section as two lines, the outer one a basement membrane. Internally, there is also a double separating membrane between the sheath and the nerve cell—resolved at the lower end of fig. 5 (Pl. 2)—so that the satellite nuclei are not, as has been stated, embedded in nerve cell substance. The boundary between sheath and nerve cell is, however, highly irregular, with folds, loops, involutions, and evaginations of nerve cell substance into the satellite cytoplasm, as though 'streamers' of various sizes and shapes were flowing out from the nerve cell surface. Some of the coiled tubular projections cut in section look like vacuolated mitochondria in the satellite cytoplasm (Pl. 2, fig. 4), and other prominent protrusions might well represent the so-called subcapsular dendrites which have been shown in silver-stained preparations. Although for the most part the cells of spinal ganglia occur singly, each with its own coverings, pairing is not infrequent, and in some of the cell pairs, parts of the adjacent cell surfaces are in immediate contact, with no intervening satellite sheath (Pl. 2, fig. 3). Palay (1957) shows electron micrographs of cell pairs, each cell, however, with a covering of satellite cytoplasm and the two sheaths separated by an extracellular cleft.

Immediately outside the satellite sheath there are Schwann cells with unmyelinated axons in their substance. The Schwann cells are usually in close contact with the satellite sheath, and indeed at light microscopic level it would be difficult, if not impossible, to distinguish one from the other. They are, however, separated from the satellite sheath by two double membranes, their own plasma cell membrane, that of the satellite cells, and the two corresponding basement membranes (Pl. 2, fig. 5). The Schwann cells do not form a continuous enclosing covering, and there are areas of cell surface with only satellite sheath (Pl. 1, fig. 2). The distribution of Schwann cells appears to be determined by the course of the axon of any particular cell which, as shown by the study of silver-stained preparations with the light microscope, varies considerably. The axon may form a 'glomerulus' to one or other side, at the upper or lower pole of the cell, or may 'festoon' the whole cell. Thus the

main axon may be cut transversely, obliquely, and/or longitudinally in any one section. The number of unmyelinated nerve fibres in the Schwann cells varies, the size range extending from fibres of the same diameter as the cell axon (4μ) to those of 0.2μ or less (Pl. 5, fig. 9), all of them closed off from the Schwann cell cytoplasm by double membranes. Because of this, the Schwann cell cytoplasm, including in some sections the adjacent satellite cytoplasm, is cut up into irregular patterns by a network of double lines. The larger axons, with their characteristic texture and mitochondria, are obvious, but elsewhere the random criss-cross network of double lines makes it difficult to distinguish between fine nerve fibres and territories of Schwann cell cytoplasm. Other Schwann cells have, in addition to a large axon, what could be small nerve fibres around the periphery, an arrangement not unlike that of the unmyelinated nerve fibres of peripheral nerve, although there are no clearly identifiable mesaxons (Pl. 5, fig. 9). This disorderly reticulation is a constant feature of the pericellular Schwann cells associated with the axon glomerulus and, it is suggested, is caused by a plexus of fine nerve fibres within these Schwann cells which overflows into the adjoining satellite cytoplasm (Pl. 3, figs. 6, 7). External to the Schwann cells there are connective tissue cells and collagenous fibres.

Out of all the material prepared, only one block yielded a few sections through the axon hillock with about 20μ of cell axon. The chances of obtaining similar sections from other cells were so small that the description of this region has had to be based on the appearances in the sections of this one cell which may not, of course, apply to all other ganglion cells. The sheath of satellite cytoplasm forms a collar at the junction of cell and axon, and thereafter the axon runs within its own Schwann cells. Flanking this first part of the axon and within the substance of the Schwann cells there is an irregular network of double-lined loops breaking up the cytoplasm and enclosing territories which in texture and type of mitochondria resemble the main axon (Pl. 4, fig. 8). Again, this appearance is interpreted to represent a plexus of fine nerve fibres coiled round the commencing cell axon within the Schwann cell cytoplasm, although the possibility of interdigitating cell processes cannot be excluded. The bounding membrane of the axon shown as a double line in fig. 10 (Pl. 5), like that of the parent cell, is irregular, with prolongations of axon substance into Schwann cell cytoplasm. Some of these projections are tubular, pursuing an irregular course, others are short triangular-shaped protrusions (Pl. 5, fig. 10). In the angle between the cell and the axon there is a Schwann cell with the 'pole' of its nucleus and a large nerve fibre. From its size it seems probable that this is a section through the first turn of the axon glomerulus.

DISCUSSION

The inability to distinguish between the Schwann cells accompanying the axon glomerulus and the true satellite cells at light-microscopic level accounts for the confusion arising from the different descriptions of the capsular elements of spinal ganglion cells. The relationship of satellite cells and nerve cell is comparable to that of axon and Schwann cells. The Schwann cells found outside the satellite sheath are associated with the axon glomerulus and are not primarily forming a capsule for the ganglion cells. The significance of the irregular nerve cell surface is not at

all clear. Similar but less elaborate folding of the limiting membranes of contiguous cells has been noted elsewhere, e.g. epithelial cells of the mucous membrane of the large intestine, and is most simply explained as a mechanism for fixing the cells together. Palay (1957) thinks the increased cell surface is necessary for the adequate nutrition of the neuron. Certainly the nearest blood vessels are extracapsular and the required substances have to diffuse through connective tissue elements as well as satellite sheath. Whatever its real meaning, the close fit of the satellite cytoplasm on to the irregular cell contour makes it unlikely that the clear space between the neuron and the capsule, seen in haemalum and eosin specimens, separates the nerve cell from the true satellite cells.

While the large axons in the Schwann cells are about the same diameter as the cell axon at its commencement, there are other axons less than half this size. While it is possible that the thickness of the axon changes within the glomerulus, it may, on the other hand, divide into two or more main branches, to rejoin before its bifurcation into peripheral and central processes.

Ehrlich (1886), Cajal (1890) and Dogiel (1896) have described a pericellular plexus of unmyelinated fibres round spinal ganglion cells, particularly plentiful, they state, in those of the horse, and less so in healthy human ganglia. According to Cajal, the pericellular plexus is prominent in diseased, injured, or transplanted ganglia, probably due to regenerative sprouting, and De Castro (1931) states that the pericellular plexus is extracapsular in position. If it is accepted that the network of double lines in the Schwann cell cytoplasm is due to a fine fibred nerve plexus invaginating into the cell substance, then there is the question of the origin of the feeding fibres of the plexus. Do they arise from the axon prior to its bifurcation? From the cell surface? From neighbouring neurons? Or from a combination of any or all of these sources?

While some of the tubular prolongations of the nerve cell surface could be regarded as dendrites, they were never seen to penetrate the satellite cytoplasm or connect up in any way with the network of the plexus. The irregularity of the axolemma of the proximal axon makes it difficult to be certain that definite branching exists. There is, however, at least one projection which could well be the origin of a branch to the surrounding plexus (Pl. 5, fig. 10). In another section (not shown) there was a short length of axon about 1μ in diameter in its Schwann cell which divided into three branches at one end. Again, the plexus is most marked within the Schwann cells associated with the axon glomerulus. What evidence there is would thus seem to indicate that the pericellular plexus is formed by branches from the axon prior to its bifurcation. The plexus is not necessarily coiled round the whole nerve cell as shown in the classical Cajal preparations, but follows the course of the axon glomerulus, and so will vary in its relation to the cell surface in the different types of ganglion cell. The question of contributions from other neurons is unlikely to be settled by electron-microscope studies. It has always been assumed that the nerve impulses pass unchanged through the spinal ganglia, although Gasser (1955) has demonstrated that 'the pattern of impulses in the nerve continues in the roots, with conduction velocities of its components reduced to between 50 and 60 % of their nerve values'.

There was no evidence of anything in the nature of a true synapse between the

cell surface and surrounding nerve fibres, but whatever the origin, the existence of a pericellular plexus must have some purpose in terms of neural activity and could for instance have a phasic effect on the mainstream of afferent impulses.

SUMMARY

The capsule of spinal ganglion cells consists of satellite cells closely adherent to the irregular surface of the nerve cell. External to this there are Schwann cells associated with the axon glomerulus, which have a varying relation to the parent nerve cell. The Schwann cells contain a size range of axons, including what is interpreted as a plexus of fine fibres. The 'pericellular' plexus is also seen in the satellite cell, and it is suggested is formed by fine branches from the pre-bifurcation part of the axon.

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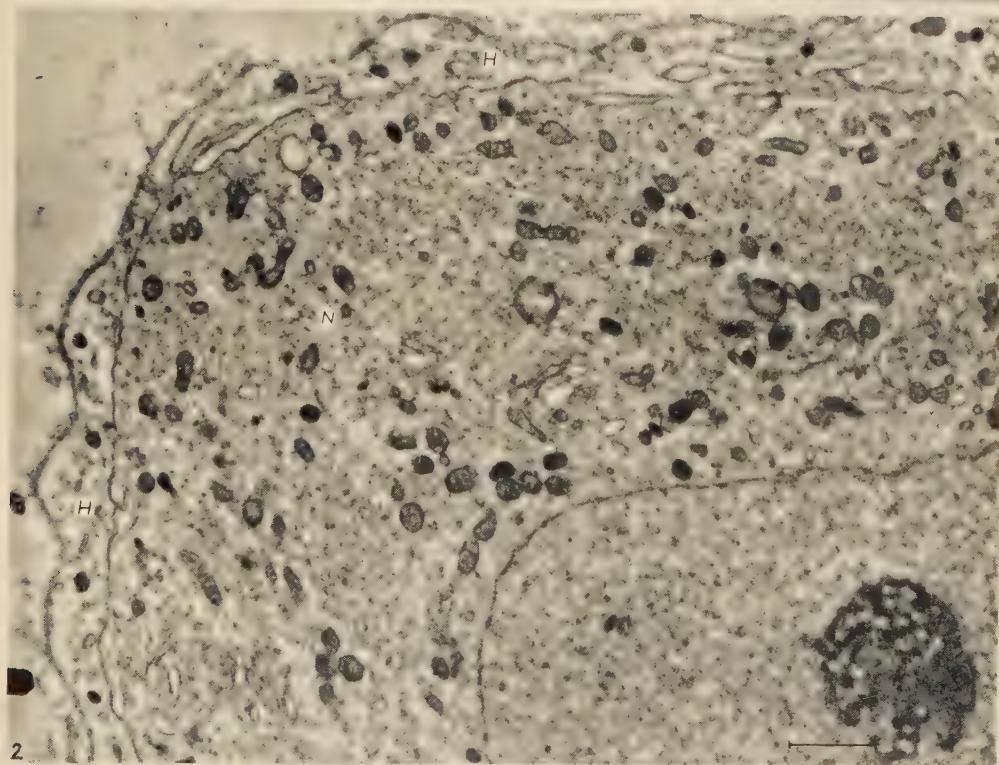
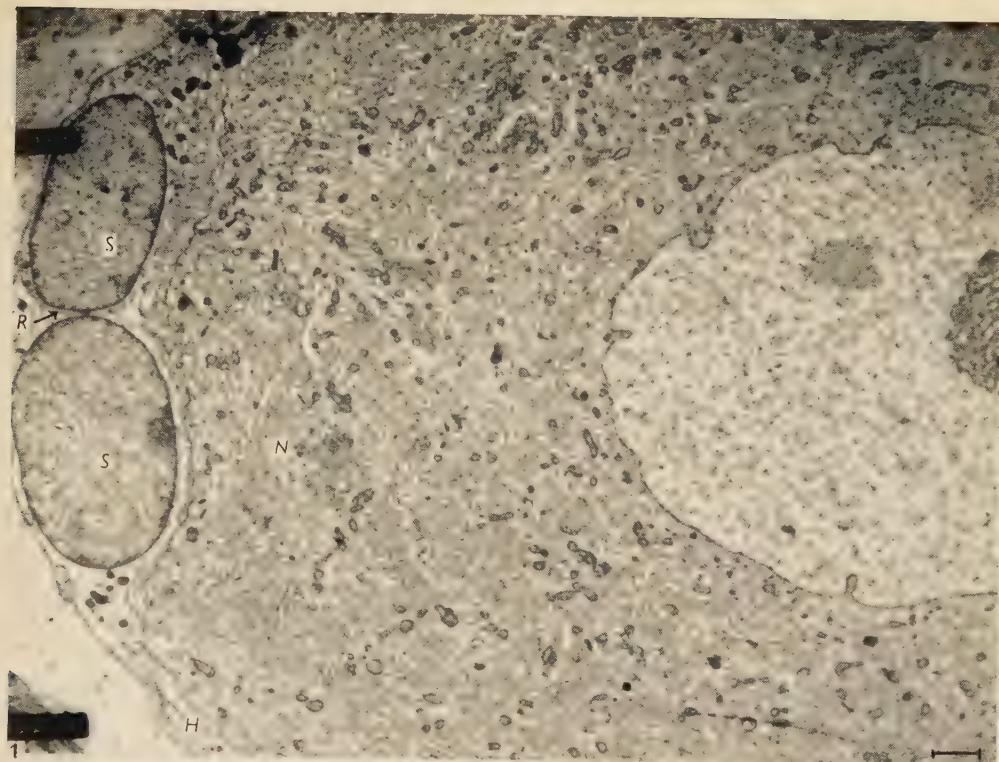
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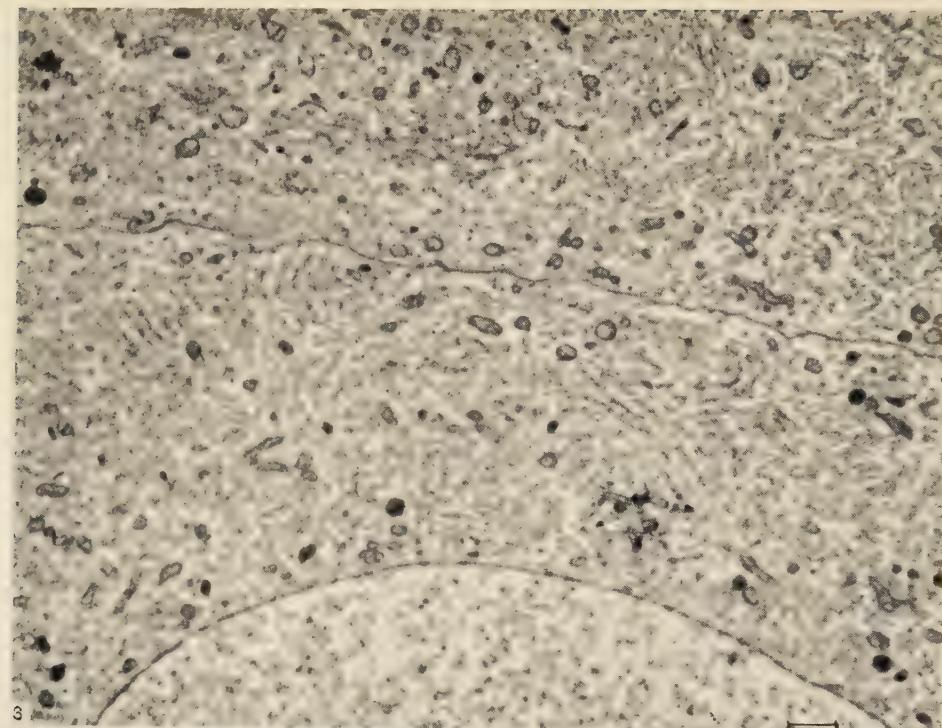
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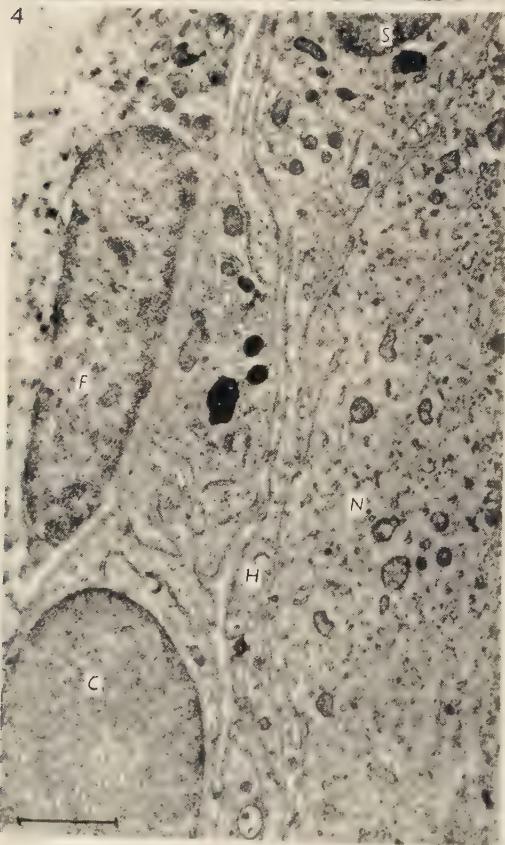
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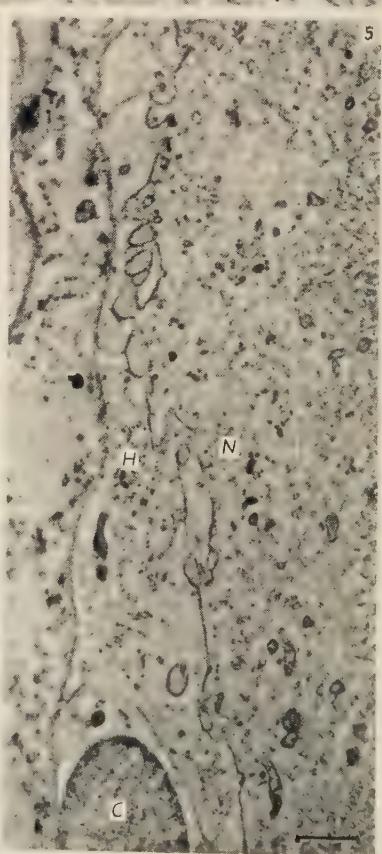




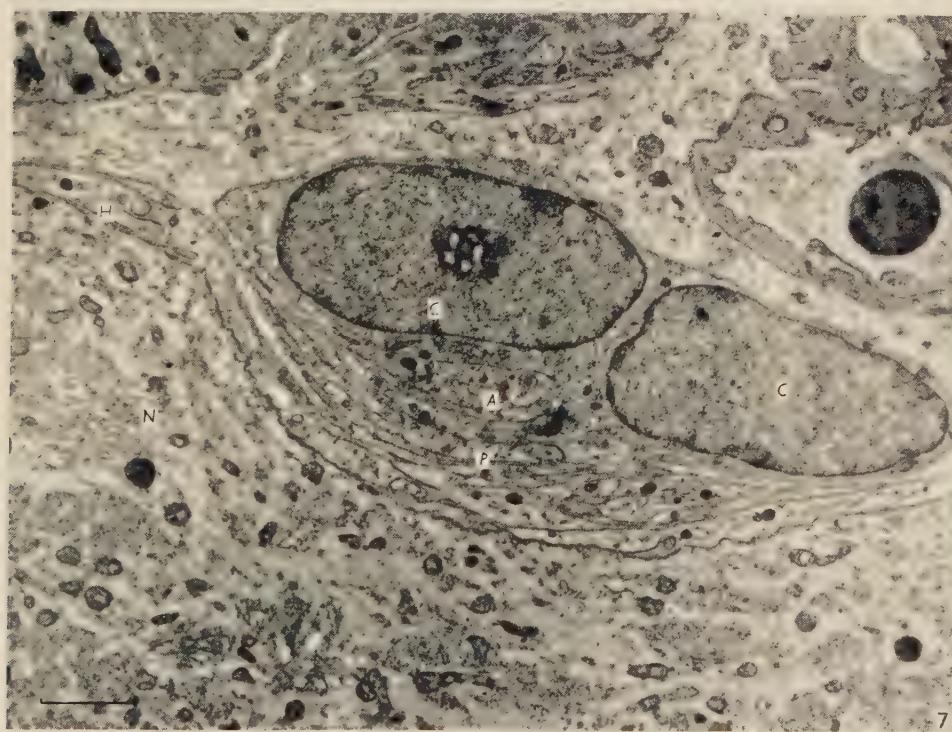
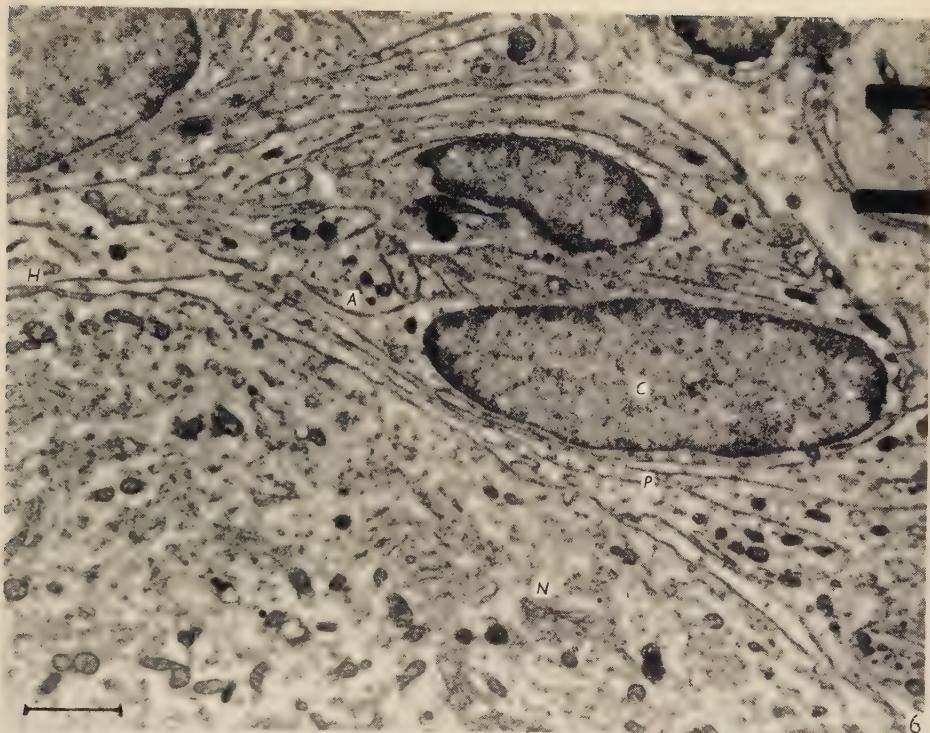
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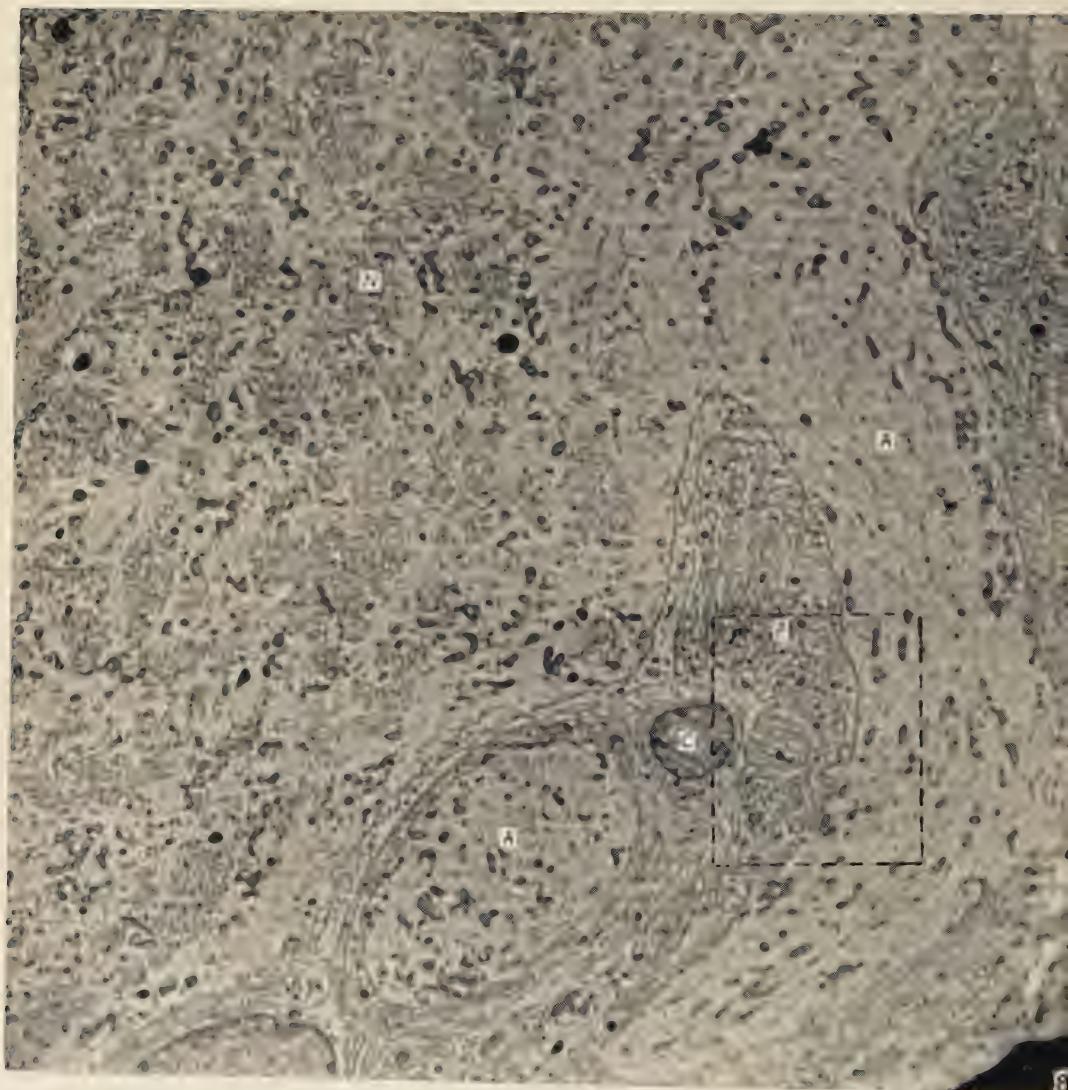


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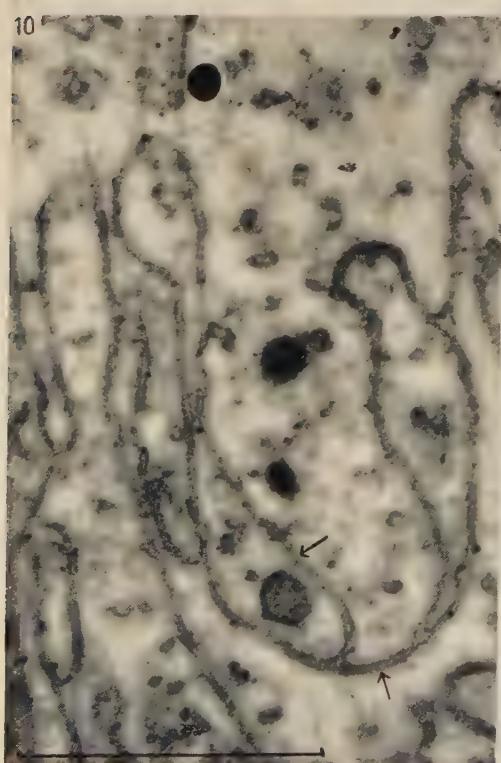
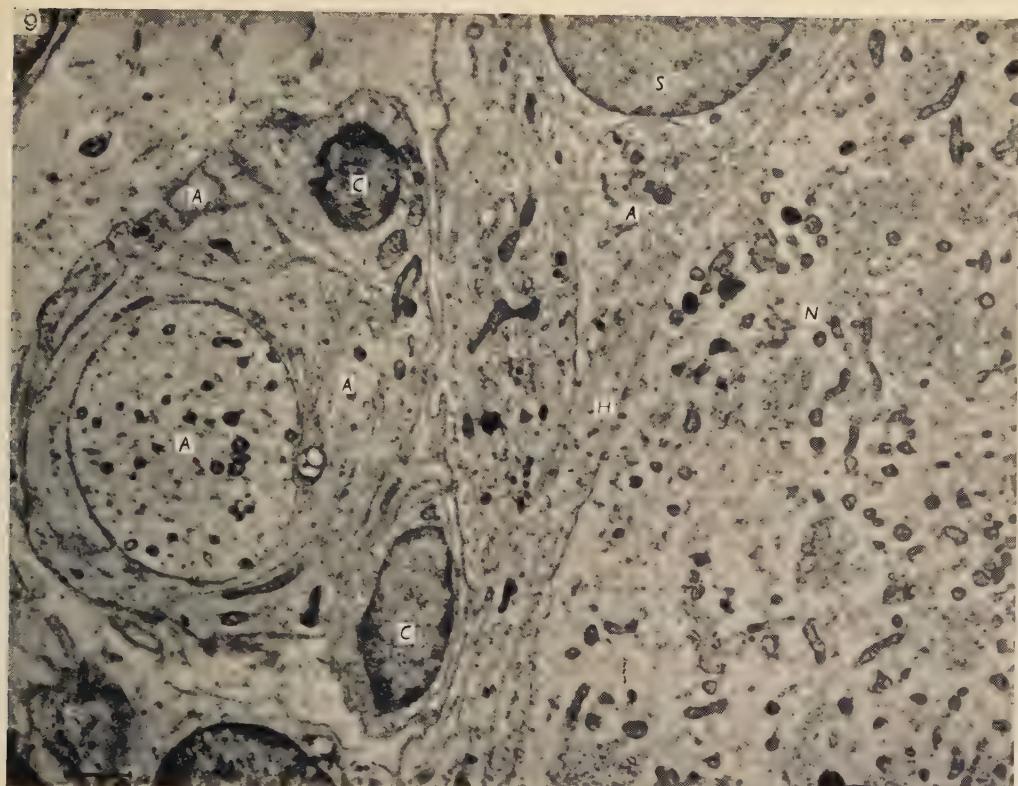


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WYBURN—THE CAPSULE OF SPINAL GANGLION CELLS



EXPLANATION OF PLATES

All figures are electron micrographs. *A*, axon; *F*, fibroblasts; *N*, nerve cell; *R*, intercellular membrane; *C*, Schwann cell nucleus; *H*, satellite sheath; *P*, pericellular plexus; *S*, satellite nucleus. The marker in each figure represents the length of one micron.

PLATE 1

Fig. 1. Section of nerve cell showing satellite cells with their nuclei.

Fig. 2. Section of nerve cell showing non-nucleated part of satellite sheath and the irregular folding and protrusion of the nerve cell surface.

PLATE 2

Fig. 3. A pair of nerve cells with no intervening satellite cytoplasm.

Fig. 4. Section of nerve cell with irregular surface and satellite sheath.

Fig. 5. Section of nerve cell with satellite sheath, Schwann cell, and fibroblast.

PLATE 3

Fig. 6. Section of nerve cell with Schwann cells, showing axons and pericellular plexus.

Fig. 7. Section of nerve cell with Schwann cells, showing axons and pericellular plexus.

PLATE 4

Fig. 8. Section of nerve cell in region of axon hillock, showing commencing axon surrounded by pericellular plexus.

PLATE 5

Fig. 9. Section of nerve cell, satellite sheath, and Schwann cells with a size range of axons.

Fig. 10. Higher magnification of area indicated in fig. 8. Note irregular axolemma and the double lines enclosing pericellular plexus.

Fig. 11. Higher magnification to show the double lines of invaginating axons.

**THE HISTOCHEMICAL APPEARANCES
OF CHOLINESTERASE IN THE PARASYMPATHETIC
NERVES SUPPLYING THE SUBMANDIBULAR AND
SUBLINGUAL SALIVARY GLANDS OF THE RAT**

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One of the greatest difficulties confronting research workers in the study of the innervation of the secretory units of the salivary glands is the extremely complex structure of these organs and the fact that this varies from species to species. Stormont (1928) and Boeke (1934) carried out a number of histological studies using silver impregnation methods in an attempt to trace the fine terminal branches of the nerves to the separate groups of cells. Unfortunately, with the silver methods it is sometimes extremely difficult to distinguish between fine nerve fibres and reticular fibres and it is impossible to classify nerve fibres as sympathetic or parasympathetic. Histological studies after degenerative section of one of the two glandular nerves might have provided the desired information. The silver methods, however, tend to give variable results which makes accurate comparative studies unreliable. Furthermore, in many instances it is extremely difficult anatomically to cut all the postganglionic parasympathetic fibres to a salivary gland.

The observation by Snell & Garrett (1957) that a histochemical method of demonstrating cholinesterase could be adapted to show the autonomic nerves within the salivary glands of the rat provided a new approach to the problem. In addition, the absence of cholinesterase in reticular fibres ruled out the possibility of confusing reticular fibres with the terminal branches of nerves. Snell & Garrett (1958) were able to trace the nerve fibres into the submandibular and sublingual salivary glands and found that they supplied the duets, arteries and all the different secretory units of the glands. Postganglionic sympathectomy was seen to have no effect on the distribution of the nerves and it was concluded that they were parasympathetic although it was pointed out that a few of the fibres might possibly be sensory. The present work is a continuation of this histochemical study and includes an investigation into the effect of ablation of the chorda tympani and lingual nerves on the distribution of the nerves within the submandibular and sublingual salivary glands. It was hoped that the results would establish with some degree of certainty the course and distribution of the parasympathetic nerves supplying these two salivary glands.

MATERIALS AND METHODS

Eighteen mature albino rats were used for the investigation. Twelve of the animals were used to study the effect of ablation of the chorda tympani and lingual nerves on the histochemical appearances of cholinesterase in the nerves within the glands.

The right lingual nerve of each animal was exposed under ether anaesthesia. With the aid of a dissecting microscope the lingual nerve was carefully defined and removed from the point where it crosses the duct of the submandibular gland to where it is joined by the chorda tympani. In addition the entire length of the chorda tympani in the infra-temporal fossa was avulsed. The animals were killed in groups of two at 10, 17 and 24 days after the operation and in groups of three at 28 and 35 days after the operation. The animals were killed by breaking the neck and the submandibular and sublingual glands on both sides were removed. In the rat the submandibular and sublingual salivary glands on each side lie in close apposition to one another and are bound together with connective tissue. This enables one to cut sections containing parts of the two glands which can then be processed at the same time under identical conditions.

The six remaining animals were used to study the extra glandular course of the nerve fibres. A bilateral block dissection of the submandibular region was performed in each animal shortly after death and the submandibular duct and sublingual duct (which run alongside one another in the rat) and the lingual nerve and the intervening tissues were removed. At the same time a short length of the chorda tympani was carefully exposed and excised.

The salivary glands of the first group of animals and the salivary ducts, lingual nerves and chorda tympani nerves of the second group were fixed in 10% neutral formol saline for 6 hr. Frozen sections 12μ thick were cut and washed in distilled water for 12 hr. The sections of the salivary ducts and the lingual nerve which had been removed *en bloc* were cut in such a way as to show either the submandibular duct or sublingual duct and the nerve in the same plane and to demonstrate the tissues which lie between them. Longitudinal frozen sections 12μ thick were cut of the chorda tympani under direct vision through a low-power microscope. All the sections were then processed using the histochemical technique described by Snell & Garrett (1957) and Snell (1958). The sections were incubated at 37°C . for 18–72 hr with the substrate solution at a pH 4.2.

To distinguish between true and pseudo cholinesterase the two substrates acetyl thiocholine iodide and butyryl thiocholine iodide were used. Acetyl thiocholine iodide is hydrolysed by both cholinesterases and butyryl thiocholine iodide is hydrolysed more rapidly by the pseudo cholinesterase. In addition, a number of the gland sections were treated with substrate solutions also containing the following inhibitors: (1) Eserine $3 \times 10^{-5}\text{M}$, to inhibit the activity of the cholinesterase group of enzymes while the simple esterases are practically insensitive. (2) B.W. 284 [1-5-bis (4-allyl-dimethylammonium phenyl) pentan-3 one dibromide] $3 \times 10^{-5}\text{M}$, to inhibit true cholinesterase. (3) DFP [di-isopropylfluorophosphate] $3 \times 10^{-10}\text{M}$, to produce relatively selective inhibition of pseudo cholinesterase. Sections were placed in an aqueous solution of the inhibitor at room temperature for 30 min. before incubation with the substrate plus the inhibitor at 37°C .

The nerves supplying the two salivary glands were identified and traced to the various groups of cells by studying the distribution of the copper sulphide precipitate which is deposited within and on the surface of nerve fibres as the result of the activity of cholinesterase present in the axons and the neurilemmal sheaths (Koelle, 1955; Snell, 1957 *a, b*). To assess the effect of ablation of the chorda tympani

and lingual nerves on the distribution of the nerves within the glands, sections from the glands of the operated side of each animal were compared with those of the control glands of the other side, the sections having been incubated with the same substrate for exactly the same length of time.

HISTOLOGICAL NOTES

The following histological notes are considered necessary in relation to the text. Stormont (1928) divided the different types of secretory cells found in mammalian salivary glands into three groups: (1) mucous cells which produce mucus, (2) sero-zymogenic cells which resemble in structure the pancreatic serous cells, and (3) special serous cells. The special serous cells can be subdivided into the tropochrome and homeochrome cells using Bensley's classification (1908). The cytoplasm of the tropochrome cells, after fixation in a formalin bichromate mixture, gives a metachromatic staining reaction with thionine. The cytoplasm of the homeochrome cells contains small granules which do not give this metachromatic reaction. In the rat the submandibular salivary gland is composed of a mixture of tropochrome and homeochrome cells, the former predominating. The sublingual gland is composed of mucous cells, and some sero-zymogenic demilune cells are also present.

RESULTS

Cholinesterase appearances in the nerves of the normal salivary glands

In previous investigations (Snell & Garrett, 1957, 1958) cholinesterase was found to be present in the nerve trunks accompanying the inter- and intralobular ducts of the submandibular and sublingual salivary glands of the rat, but the activity of the enzyme appeared to be less in the nerves supplying the sublingual gland. True and pseudo cholinesterase activity was present in the nerve trunks, but the pseudo cholinesterase was mainly concentrated in the connective tissue of the nerves. The nerves were seen to give branches to the ducts and arteries and terminated by breaking up to form a network which surrounded the secretory acini. In the submandibular gland the arrangement of the nerve net in the vicinity of the tropochrome and homeochrome cells was practically identical. In the sublingual gland fewer nerve fibres were visible but their arrangement in the region of the mucous and demilune cells appeared to be the same. In addition, cholinesterase activity was detected in small amounts in the region of the walls of the secretory cells, especially in those of the tropochrome type. Some enzyme activity was also seen in the nuclei and cytoplasm of many of the cells of the inter- and intralobular ducts.

In the present work in the normal glands the above findings were confirmed.

Cholinesterase appearances in the nerves of the salivary glands following ablation of the chorda tympani and lingual nerves

In all the sections of the salivary glands which had undergone ablation of the chorda tympani and lingual nerves the overall picture of the cholinesterase activity in the nerves, including the main trunks, the branches to the ducts and blood vessels, and the branches to the secretory acini was practically identical to that seen in the

control gland sections (Pl. 1, figs. 1-6; Pl. 2, fig. 7). Furthermore, this operation appeared to have no effect on the enzyme activity in the region of the walls of the secretory cells or in the nuclei and cytoplasm of the cells of the inter- and intra-lobular ducts.

*Cholinesterase appearances in the extra-glandular nerves and ganglia
in the submandibular region and infra-temporal fossa*

Chorda tympani

In the longitudinal sections of the chorda tympani that had been incubated with acetyl thiocholine iodide for 48 hr. the majority of the nerve fibres showed very little cholinesterase activity. In some of the sections, however, a small compact group of fibres showed a high concentration of copper sulphide precipitate indicating a large amount of cholinesterase activity. Lying along the course of these fibres dark fusiform expansions were noted which contained a very high concentration of copper sulphide precipitate (Pl. 2, fig. 8). In the sections which had been incubated for 24 hr. it was noted that these expansions were composed of ganglion cells and nerve fibres.

Lingual nerve

In the sections of the lingual nerve which had been incubated with acetyl thiocholine iodide for 48 hr. the majority of the nerve fibres showed very little cholinesterase activity. A careful examination of some of the sections in the region distal to the point where the lingual nerve is joined by the chorda tympani revealed a number of fibres which showed a high concentration of copper sulphide precipitate (Pl. 2, fig. 9). It was seen that these fibres had an identical appearance to those seen in the chorda tympani and had presumably entered the lingual nerve from the chorda tympani. Here again an occasional dark fusiform expansion was noted along the course of these fibres. In the sections showing the lingual nerve winding round the ducts of the submandibular and sublingual salivary glands the fibres possessing a large amount of cholinesterase activity were seen to leave the lingual nerve and pass across to the ducts, where they divided up to form a series of intercommunicating nerve bundles which were embedded in the connective tissue coat of the ducts (Pl. 2, fig. 10). A further series of dark fusiform expansions was seen along the course of the nerve fibres as they traversed the connective tissue between the lingual nerve and the ducts, and as they accompanied the ducts to the submandibular and sublingual salivary glands (Pl. 2, figs. 10, 11).

Ganglia

These were seen as a series of fusiform swellings along the course of the nerve fibres showing a high cholinesterase activity and were situated in the chorda tympani, lingual nerve and in the connective tissue coat of the submandibular and sublingual ducts. The majority of the ganglia were found along the course of the fibres after they had left the lingual nerve. In the sections which were incubated for 48 hr. the ganglia appeared black due to the presence of a high concentration of copper sulphide precipitate and no structural detail could be made out. In the

sections which were incubated for 24 hr. the ganglia were seen to consist of large rounded cells (Pl. 2, fig. 12). A fine deposit of copper sulphide precipitate was seen to be present within the nucleus and a very fine deposit was also seen in the cytoplasm of the cells. Lying insinuated between the ganglion cells were slender nerve fibrils which contained a high concentration of copper sulphide precipitate. A number of the fibrils showed dark expansions along their course in the region of the ganglion cells suggestive of boutons terminaux or boutons de passage.

DISCUSSION

The question as to whether the parasympathetic and sympathetic nerves innervate different cells or the same cell in the salivary glands has been the subject of much discussion for over half a century. Langley (1889, 1890) believed that the parasympathetic and sympathetic fibres innervate different cells in the glands and this opinion was shared by Gaskell (1920), Stormont (1926, 1928), Rawlinson (1933, 1935) and Babkin (1950). Heidenhain (1868) on the other hand considered that each secretory cell is controlled by two types of nerve fibres. Langenskiöld (1941) also held this view as the result of electrophysiological experiments carried out on the submandibular gland of the cat. Emmelin (1955a, b) came to the same conclusions following a study of the flow of submandibular salivary secretion in response to nerve stimulation in the same animal. Moloy & Smith (1930) on the basis of histological findings in the submandibular gland of the rabbit following parasympathetic and sympathetic stimulation, considered that both the tropochrome and homeochrome cells were influenced by the parasympathetic, but the sympathetic seemed to have no effect on either of these cells. It is clear that in spite of all these studies the precise innervation of the cells of the salivary glands remains obscure.

Snell & Garrett (1958), reporting on the histochemical appearances of cholinesterase in the nerves supplying the submandibular and sublingual salivary glands in the rat, found that postganglionic sympathectomy had no effect on the distribution of the nerves to the ducts, arteries and all the different secretory cells of the glands. It was considered that the nerves seen within the glands were parasympathetic, although it was appreciated that a number of the fibres might be sensory.

The results of the present work in the normal glands of the rat confirm previous findings (Snell & Garrett, 1957, 1958). The operative procedure in which the chorda tympani and lingual nerves were ablated may be regarded as preganglionic parasympathectomy, since the results showed that the majority of the parasympathetic ganglia are situated along the course of the nerve fibres, after they have left the lingual nerve. This operation, however, unavoidably resulted in the removal of a small number of parasympathetic ganglia lying within the chorda tympani and lingual nerves and thus a few postganglionic fibres underwent section and presumably degenerated. Nevertheless, since the appearances of the copper sulphide precipitate in the nerve fibres lying within the salivary glands on the operated side were seen to be practically identical to those found on the control side, it may be concluded that preganglionic parasympathectomy has no effect on the activity of cholinesterase in the postganglionic parasympathetic fibres. This finding was to be

expected since Langley (1898) showed that section of the chorda tympani in the cat has no effect on the structure of the postganglionic neurones.

The observation that the majority of the nerve fibres present in the chorda tympani and lingual nerves, which are almost certainly sensory in nature, possess very little cholinesterase activity is in agreement with the findings of Koelle (1955) who showed that sensory fibres possess a lower concentration of cholinesterase than fibres of cholinergic neurones. In view of this it is very unlikely that any sensory nerve fibres were seen in the sections of the salivary glands either in the present research or in previous work (Snell & Garrett, 1957, 1958) when the same histochemical method was used to demonstrate the nerves.

The small compact group of nerve fibres showing a large amount of cholinesterase activity in the chorda tympani and lingual nerves and possessing ganglia along their course are presumably the parasympathetic fibres, since parasympathetic fibres are cholinergic and are known to possess a high concentration of cholinesterase, whereas sympathetic adrenergic fibres possess a lower concentration of cholinesterase (Koelle, 1955). Langley (1890) described the arrangement of the parasympathetic nerve fibres running from the chorda tympani to the sublingual and submandibular glands of the dog. He found that a large number of small ganglia exist in the connective tissue triangle lying between the chorda tympani, lingual nerve and submandibular duct. He reported that the majority of these ganglia occurred on the course of nerve fibres bound for the sublingual gland and that comparatively few of the nerve fibres for the submandibular gland have nerve cells on their course before they enter the submandibular gland.

The present work on the rat shows the arrangement of the parasympathetic ganglia to be similar to that of the dog in that they are small and numerous and many are scattered along the course of the nerve fibres in the connective tissue which lies between the lingual nerve and the submandibular and sublingual ducts. It was not possible to determine which ganglion or group of ganglia control a particular salivary gland. It was noted, however, that in some of the sections of the submandibular gland ganglion cells were seen to be present in close association with the nerve trunks accompanying the main ducts deep inside the gland, and the function of these cells is almost certainly directed exclusively to controlling the submandibular gland. No ganglion cells were found within the sublingual salivary gland.

These results, taken together with those already reported on the effect of post-ganglionic sympathectomy, would indicate that the nerve fibres which can be demonstrated by this method in the normal submandibular and sublingual salivary glands of the rat are parasympathetic. It would thus appear that parasympathetic nerve fibres supply the ducts and arteries of both the salivary glands. In the submandibular gland the parasympathetic also supplies the homeochrome and tropochrome cells while in the sublingual gland they supply the mucous and demilune cells. These conclusions are in agreement with the general beliefs of Moloy & Smith (1930) for the rabbit, Langenskiöld (1941) and Emmelin (1955a, b) for the cat, but contrast markedly with the findings of Stormont (1928) who reported that in the submandibular gland of the rabbit anatomically and functionally the homeochrome cells are supplied by the parasympathetic fibres, and the tropochrome cells by the

sympathetic fibres. No evidence could be found in the present research of nerve fibres ending on the secretory cells in the form of end bulbs or varicosities as described by Stormont (1928).

The existence of cholinesterase activity in the nuclei and cytoplasm of the parasympathetic ganglion cells found in the chorda tympani, lingual nerve and in the connective tissue in the region of the ducts of the salivary glands is of interest. Koelle (1951) reported that in the ciliary ganglion of the cat high concentrations of true cholinesterase activity are present in the cytoplasm of the ganglion cells and their processes and that pseudo cholinesterase is found in the capsular cells. No enzyme activity was, however, seen in the ganglion cell nuclei. In the present research no attempt was made to distinguish between true and pseudo cholinesterase activity within the ganglia.

The functional role of cholinesterase in nerve cells and their processes is not exactly understood. Hollinshead & Sawyer (1945) have correlated the presence of high concentrations of true cholinesterase found in cholinergic neurones with the presence of relatively high amounts of acetylcholine (MacIntosh, 1941). Lissák (1939) has shown that during the conduction of nerve impulses along a cholinergic axon acetylcholine is liberated along its entire length. These findings have been interpreted as indicating that the acetylcholine-cholinesterase mechanism is in some way related to the process of nerve conduction. Considerable evidence against this theory has, however, been reviewed by Feldberg (1945) and Koelle & Gilman (1949).

The presence of cholinesterase activity in the region of the walls of the tropochrome and homeochrome cells, but more especially those of the tropochrome cells, is also of interest. It is possible that the activity of this enzyme in these locations is intimately related to the passage of substances to and from the cells through the cell walls. The presence of cholinesterase in the nuclei and cytoplasm of the duct cells of the inter- and intralobular ducts confirms previous findings (Snell & Garrett, 1957, 1958) and is possibly associated with a secretory or absorptive function.

SUMMARY

1. The innervation of the submandibular and sublingual salivary glands of the rat has been studied histologically. A histochemical method for cholinesterase was used to demonstrate the nerve fibres.
2. The nerves were found to enter the glands as a series of intercommunicating trunks which accompanied and gave branches to the ducts and arteries. The nerves were seen to terminate by breaking up to form a network which supplies all the secretory cells of the glands.
3. Ablation of the chorda tympani and lingual nerves was found to have no effect on the histochemical appearances or distribution of the nerves within the glands.
4. In the chorda tympani and lingual nerves the sensory fibres were seen to have very little cholinesterase activity. The small compact group of parasympathetic fibres and the ganglion cells scattered along their course, however, showed a very high cholinesterase activity.
5. The results of the present investigation taken in conjunction with those

obtained after postganglionic sympathectomy reported previously, indicate that all the nerve fibres demonstrated within the salivary glands by this method are parasympathetic.

6. The significance of these findings in relation to histological and physiological observations of past and present workers is discussed.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photomicrograph of section of large duct of submandibular salivary gland of rat. Shows black copper sulphide precipitate in the nerves supplying the duct. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 270$.

Fig. 2. Photomicrograph of section of artery of submandibular salivary gland of rat. Shows black copper sulphide precipitate in the nerves supplying the artery. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 350$.

Fig. 3. Photomicrograph of section of submandibular salivary gland of rat. Shows black copper sulphide precipitate in the fine nerve fibres around the homeochrome and tropochrome cell acini. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 142$.

Fig. 4. Photomicrograph of section of submandibular salivary gland of rat which had undergone ablation of the chorda tympani and lingual nerves 28 days previously. Shows black copper sulphide precipitate in the nerve trunks accompanying an interlobular duct. The precipitate was formed as the result of cholinesterase activity and appeared to be identical to that seen in the nerves of sections of the control gland. Acetyl thiocholine iodide was used as substrate. $\times 58$.

Fig. 5. Photomicrograph of section of large duct of submandibular gland of rat which had undergone ablation of the chorda tympani and lingual nerves 24 days previously. Shows black copper sulphide precipitate in the nerves supplying the duct. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 234$.

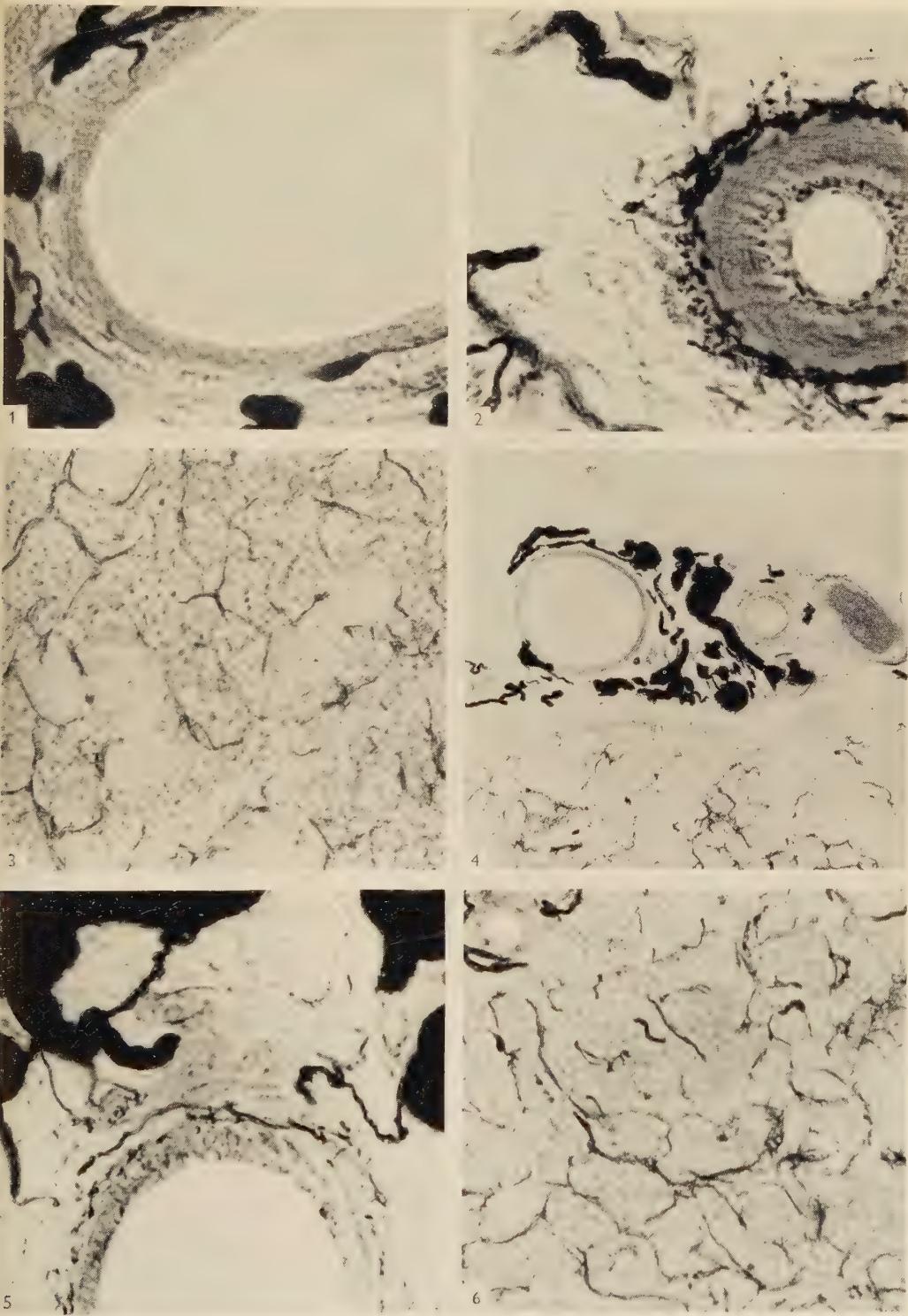
Fig. 6. Photomicrograph of section of submandibular salivary gland of rat which had undergone ablation of the chorda tympani and lingual nerves 28 days previously. Shows black copper sulphide precipitate in the fine nerve fibres around the acini. The precipitate was formed as the result of cholinesterase activity and appeared to be identical to that seen in the nerves of sections of the control gland. Acetyl thiocholine iodide was used as substrate. $\times 160$.

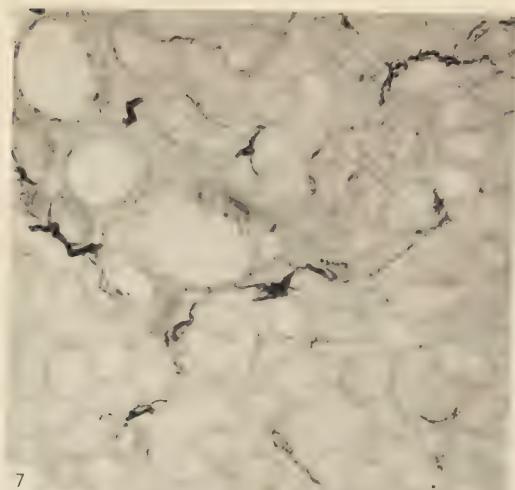
PLATE 2

Fig. 7. Photomicrograph of section of sublingual salivary gland of rat which had undergone ablation of the chorda tympani and lingual nerves 35 days previously. Shows only a small amount of copper sulphide precipitate in the fine nerve fibres around the acini. The precipitate was formed as the result of cholinesterase activity and appeared to be identical to that seen in the nerves of sections of the control gland. Acetyl thiocholine iodide was used as substrate. $\times 140$.

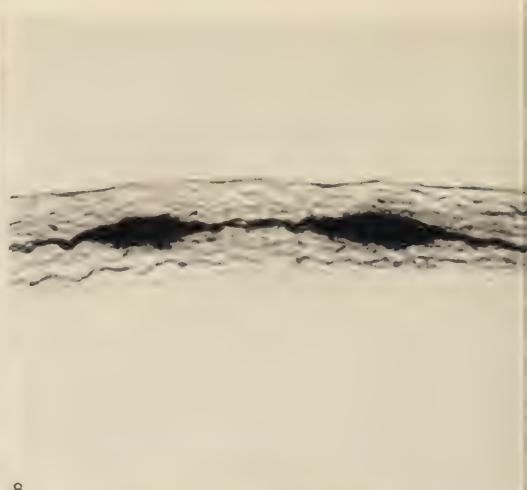
Fig. 8. Photomicrograph of section of chorda tympani nerve of rat. Shows a small number of fibres and two parasympathetic ganglia containing a large amount of black copper sulphide precipitate. The remaining nerve fibres show only a small amount of copper sulphide precipitate. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 190$.

Fig. 9. Photomicrograph of section of lingual nerve of rat distal to the point where it has been joined by the chorda tympani. A large amount of black copper sulphide precipitate is seen in the nerve fibres which have come from the chorda tympani and are situated in the upper part of the nerve. The remaining fibres show very little precipitate. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 207$.





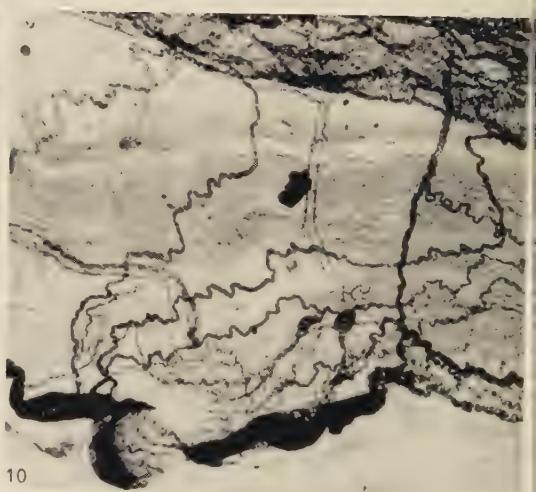
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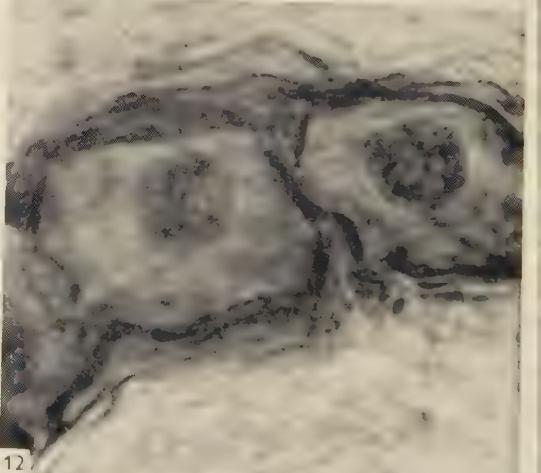
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Fig. 10. Photomicrograph of section of lingual nerve and submandibular duct of rat. The lower margin of the duct is seen in the upper part of the photograph and the lingual nerve occupies the centre background and is about to cross behind the duct. A large amount of black copper sulphide precipitate is seen in the fine parasympathetic fibres passing from the lingual nerve to the duct. Note the large ganglia in the lower part of the photograph. The majority of the nerve fibres in the lingual nerve show only a small amount of the precipitate. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 63$.

Fig. 11. Photomicrograph of longitudinal section of submandibular duct of rat. Shows black copper sulphide precipitate in the nerve fibres in the connective tissue coat of the duct. Note the ganglion in the upper part of the picture. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 90$.

Fig. 12. Photomicrograph of section of ganglion lying on the course of a nerve fibre passing from the lingual nerve to the ducts of the submandibular and sublingual salivary glands of the rat. Shows parts of two ganglion cells and numerous fine nerve fibres. The black copper sulphide precipitate is seen to be present in the nucleus and cytoplasm of the ganglion cells and in the nerve fibres. The precipitate was formed as the result of cholinesterase activity. Acetyl thiocholine iodide was used as substrate. $\times 1350$.

THE ASSOCIATION OF ALKALINE PHOSPHATASE WITH FIBROGENESIS

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In a recent study of the healing of lesions in the gall-bladder of the cat, McMinn & Johnson (1957) were unable to demonstrate alkaline phosphatase in the new connective tissue that was formed during the repair process. This negative finding for phosphatase in new connective tissue is at variance with the results of Fell & Danielli (1943), who showed that there was a considerable increase in the amount of alkaline phosphatase associated with maturing granulation tissue during the healing of skin wounds in the rat. The present study was undertaken to determine whether these contrasting results in wound healing were due either to an organ difference, a species difference, or to some artefact arising during the carrying out of the histochemical technique.

MATERIALS AND METHODS

Fibroplasia has been studied in the cat during wound healing in the oesophagus, stomach, small intestine, rectum, urinary bladder and skin and in the rat during the repair of lesions in the rectum and skin.

Operative procedures

All operations on the cat were performed on healthy adult animals under Nembutal anaesthesia; the adult rats were anaesthetized with ether.

In the hollow viscera, the fibroplasia was produced by the removal of pieces of mucous membrane 0·5–1·0 cm.² in size, as described for the urinary bladder and oesophagus by McMinn & Johnson (1955, 1958) and for the small intestine by McMinn & Mitchell (1954). In the cat's stomach, the lesions were made by removing the piece of mucosa from the middle of the greater curvature, after incising the ventral surface of the viscus. The rectal wounds in the cat and the rat were created by removing the required area of mucous membrane *per anum*, those in the rat being only a few square millimetres in area. A total of 112 visceral lesions was available for study.

The skin wounds, on the ventral abdominal wall in both species, were produced by the removal of 0·5–1·0 cm.² of epithelium with the underlying dermis and a varying amount of subcutaneous tissue, a total of thirty being examined. The wounds were left open and no dressings applied.

In addition, five skin lesions were made in cats that had received a dietary supplement of vitamin C (100 mg. ascorbic acid daily for 2 days before operation and on each post-operative day).

Following the above operative procedures, the animals were allowed to survive for periods varying from 1 day to 6 months, the greater number being sacrificed between the 7th and 21st days, a period during which fibrogenesis was found to be most active. At the time of sacrifice the sites of the lesions were removed and immediately fixed in ice-cold 80% alcohol.

Histological and histochemical techniques

After embedding in paraffin wax, serial sections were cut at a thickness of 8μ , and every twentieth mounted for routine staining with haematoxylin and eosin. These were then examined, and from regions of particular interest serial sections were mounted for further investigation. For the demonstration of alkaline phosphatase the Gomori cobalt sulphide technique was employed. Sodium β -glycerophosphate was used as a substrate and Mg ions were added to the incubation medium to activate the enzyme as suggested by Kabat & Furth (1941). The incubation periods varied between 5 min. and 24 hr.; for routine examination, $\frac{1}{2}$ hr. and 3 hr. periods were those most frequently used. In addition the simultaneous coupling azo-dye technique of Menten, Junge & Green (1944) as modified by Manheimer & Seligman (1949) and by Gomori (1951) was used to confirm the findings with the cobalt sulphide method.

For the demonstration of the new connective tissue laid down during the healing of the various lesions, sections were stained with haematoxylin and eosin, iron haematoxylin and picrofuchsin and Gomori's and Gordon and Sweets' stains for reticulum.

Sections from each lesion were also subjected to the periodic acid-Schiff (PAS) technique. In this the acid-reducing rinse was not employed; the omission of this step enhances the demonstration of collagenous tissue, as suggested by Lillie (1953).

RESULTS

The histological findings in the healing wounds of both species were typical of any healing by granulation.

In the skin, the lesions in all animals had become completely epithelialized by the 10th day, and by this time the subepithelial tissues consisted of a mass of new fibroblasts with considerable extracellular material and numerous regenerating blood vessels (Pl. 1, fig. 1).

In the hollow viscera, the process of repair in connective tissue followed a similar pattern to that seen in the skin. By the 2nd post-operative day fibroblasts began to undergo mitosis and this activity increased until about the 7th day and thereafter declined. Extracellular material was apparent by the 10th day (Pl. 1, fig. 2).

In the rat, examination of the skin and rectal wounds for alkaline phosphatase revealed that during the 2nd and 3rd weeks, when fibroplasia was most active, the new connective tissue gave an intense reaction for this enzyme (Pl. 1, figs. 3, 4). The positive reaction could be demonstrated with incubation periods as short as 10 min. The reaction was strictly localized to the regenerating area and on close examination was seen to be present in the fibroblasts, young collagenous fibres, blood vessels and infiltrating cells. In the fibroblasts the reaction was mainly cytoplasmic. In the

normal tissue surrounding the skin lesions, all structures including the epithelium were negative with periods of incubation up to 24 hr., apart from sebaceous glands and hair follicles (Pl. 1, fig. 3). In the rectum the surrounding tissue showed some stromal reaction which was mainly confined to the blood vessels (Pl. 1, fig. 4). The positive findings with the Gomori technique were confirmed in both sites with the azo-dye method.

This positive reaction in the healing skin and rectal ulcers in the rat contrasted sharply with the findings in the cat. In lesions of the skin and hollow viscera in this species, the newly formed connective tissue, at all stages of its growth, gave a negative reaction for the enzyme, even following prolonged incubation (Pl. 2, fig. 5). In many instances, for example in the small intestine and skin, structures such as the brush border and sebaceous glands, respectively, gave an intense reaction, but the young connective tissue, which had an appearance similar to that seen in the rat, gave a completely negative response for alkaline phosphatase.

In cats receiving large doses of vitamin C no evidence was found to show that fibroplasia in their tissues differed from that in animals receiving the normal diet, and the reaction for alkaline phosphatase was still negative.

Additional experiments

In view of the present negative findings in the cat, the following experiments were carried out with the object of establishing the validity or otherwise of the positive results in the rat.

Since it could be claimed that new connective tissue in the rat might adsorb phosphatase from the tissue fluid of the body, the first experiment was performed to determine (*in vitro*) if the connective tissue of the rat had a greater affinity for the enzyme than other rat tissue elements or the tissues of the cat. The inherent phosphatase in sections of skin ulcers of the rat was inactivated either by immersion in water at 90° C. for 10 min. or in Lugol's solution for 10 min. Following this they were put into a buffered solution which contained either an homogenate of the mucosa of rat's duodenum (a rich source of the enzyme), or commercial alkaline phosphatase, for 90 min. The sections were then thoroughly washed in running water and transferred to normal substrate and incubated for 3 hr. Any cellular element that had an affinity for alkaline phosphatase might be expected to take it up and then, later during the performance of the histochemical technique, to give a positive reaction. It was found that not only the fibroblasts but also the epithelial cells showed an affinity for the enzyme, the reaction being strongest in the nuclear membranes and nucleoli (Pl. 2, fig. 6). Experiments of the same nature were carried out on sections from skin ulcers in the cat, and these gave results similar in all respects to those in the rat.

In a second experiment, performed to detect diffusion of reaction products in the histochemical technique, sections of rat-skin ulcers or of the kidney cortex of the rat were superimposed on sections from skin ulcers in which the 'native' phosphatase had been destroyed. These combined sections were then incubated for periods up to 48 hr. and taken through the remaining steps of the histochemical technique in the usual manner. On examination it was seen that all tissue elements in that part of the inactivated section adjacent to the margin of the overlying

section gave a positive reaction (Pl. 2, fig. 7), indicating that there had been a diffusion of some of the products of reaction during the histochemical procedure. However, diffusion was negligible with periods of incubation of less than 24 hr. Similar experiments performed with skin ulcers of the cat gave similar results.

A third experiment was designed to determine the affinity of the rat and cat tissues for calcium phosphate. Sections in which the phosphatase had been inactivated were incubated in normal substrate to which either hydrogen peroxide or commercial alkaline phosphatase was added. Following the incubation the sections were thoroughly washed and the remaining steps of the histochemical technique performed. In this experiment the hydrogen peroxide or the commercial alkaline phosphatase which had been added to the medium caused the formation of a precipitate of calcium phosphate in the solution. It was expected that any element having an affinity for calcium phosphate would adsorb it, and that when the sections were taken into cobalt nitrate and ammonium sulphide, a positive reaction would occur in these elements. Examination of the sections did reveal that in both species the nuclei of all cells had a considerable affinity for calcium phosphate (Pl. 2, fig. 8).

DISCUSSION

The present work shows that during all stages of wound healing in the cat, collagen fibres and fibroblasts show no evidence of phosphatase activity as demonstrated by histochemical tests. In contrast, fibroplasia in the rat is accompanied by considerable enzymatic activity.

Gold & Gould (1951) questioned the significance of the apparent relationship between high phosphatase activity and fibrogenesis. They suggested that the positive reaction for phosphatase was due to the fibrous proteins, at some stage in their formation, adsorbing the enzyme from surrounding tissue fluids and concentrating it so that histochemical methods visualize it. Biochemical tests, such as were carried out by Fell & Danielli (1943) to confirm the increase that had been demonstrated histochemically, cannot of course determine whether the phosphatase present has been adsorbed (a possibility of which those authors were aware), or whether it is 'native' to cells or fibres.

It has been seen in the present work that when sections, whose native phosphatase was destroyed, were placed in a solution containing the enzyme, the tissues in both species have an affinity for phosphatase. The affinity was shown not only by the fibroblasts in each animal, but also by such structures as the epidermis. Although there is this evidence for an adsorption of the enzyme to cells, close examination revealed that there was a distinct difference between the distribution of 'native' and adsorbed enzymes. When 'native' phosphatase was demonstrated, the reaction in fibroblasts was particularly intense in the cytoplasm, whereas when there had been adsorption the reaction was mainly confined to nuclear membranes and nucleoli. If the reaction which was seen in tissues of the healing wound in the rat were due to an adsorption of the enzyme, then it would have to be explained why the epithelial cells in the rat, and the regenerating tissue elements in the cat, always gave a negative reaction, since the cat tissues and the rat epithelium have an affinity for phosphatase equal to that of the fibroblasts of the rat.

It is well known that calcium phosphate under certain conditions can be adsorbed to cellular elements in the Gomori technique and so give rise to false positive results. Among the different factors which give rise to these false results are inadequate concentrations of calcium nitrate in the substrate solution, low pH, and excessively long periods of incubation (Ruyter & Neumann, 1949; Gomori, 1950, 1951; Novikoff, 1951; Yokoyama, Stowell & Mathews, 1951). The present authors have been conscious of these possible sources of error in the carrying out of the histochemical technique and have shown that in the present tissues diffusion of calcium phosphate from prolonged incubation does not occur until after 24 hr. It has also been shown that when the calcium phosphate is liberated by adding either the enzyme or hydrogen peroxide to the incubation medium, in the rat, fibroblasts have no greater affinity than epithelial cells for calcium phosphate, nor have the rat tissues any greater affinity than those of the cat. In addition, the final appearance following the calcium phosphate experiment differs from that seen with the normal Gomori technique.

From these observations it may be concluded that there is a true difference between the rat and the cat with regard to alkaline phosphatase activity in new connective tissue.

A number of workers, including Bourne (1944), Danielli, Fell & Kodicek (1945), Bunting & White (1950) and Robertson & Schwartz (1953), have shown that in vitamin C deficiency there is a considerable decrease in collagen formation during wound healing, and that this is associated with a lowered phosphatase content. The domestic cat on a normal diet is most unlikely to be deficient in vitamin C since, unlike primates and the guinea-pig, it can synthesize its own requirements (Burns, 1957). The results of the experiments carried out on animals that had received a dietary supplement of this vitamin were similar to those in cats that had received a normal diet, and this affords evidence that the absence of phosphatase was not due to such a deficiency.

In contrast to the work (e.g. of Danielli, Fell & Kodicek, 1945) suggesting the association between alkaline phosphatase and fibrogenesis, other investigators such as Marchant (1949) and Robertson, Dunihue & Novikoff (1950) have failed to demonstrate such a correlation. Gould & Gold (1951) who applied phosphatase to wounds of scorbutic animals and who injected phosphatase inhibitors into wounds where there was a rapid fibre formation, considered that phosphatase plays a doubtful role in fibrogenesis. Other evidence suggests: (1) that it is concerned in the differentiation of new fibroblasts, a concept which is supported by the work of Moog (1944), who showed that in the development of the chick there are waves of formation of phosphatase in the development of most tissues, and of Lorch (1949), who demonstrated similar results in the dogfish and trout; (2) that it is concerned with the secretion or formation of the precursors of collagen fibres, a suggestion which gains support by the finding of phosphatase in such structures as hair follicles and in the free border of the secreting cells in the glands of the silkworm (Bradfield, 1950); and (3) that it may be involved in the elaboration of the mucopolysaccharides of the ground substance of connective tissue (Kroon, 1952; Moog & Wenger, 1952).

The present negative findings for phosphatase at sites of fibrogenesis during

wound healing indicate that in the cat this enzyme may not be essential for the production of fibrous protein, or that the mechanisms involved may vary from one species to another.

SUMMARY

1. Fibroplasia has been studied in the cat and rat during wound healing in skin and in various abdominal hollow viscera.
2. The fibrous tissue elements formed during wound healing in skin and hollow viscera in the cat do not give a positive reaction for phosphatase, in contrast to the rat in which alkaline phosphatase is always associated with new fibrous tissue.
3. The possible roles of alkaline phosphatase in fibrogenesis are discussed, and the conclusion reached that phosphatase may not be essential for the production of fibrous protein in the cat.

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EXPLANATION OF PLATES

All photomicrographs are from specimens 10 days after operation. Sections demonstrating the presence of alkaline phosphatase have not been counter-stained.

PLATE 1

Fig. 1. Centre of a skin wound in the rat, showing young connective tissue elements underlying new epithelium. H. & E. $\times 640$.

Fig. 2. New connective tissue elements underlying new epithelium in a healing wound of the oesophagus in the cat. Note the mitotic figure in a fibroblast. H. & E. $\times 640$.

Fig. 3. Healing skin wound in the rat demonstrating alkaline phosphatase in the newly formed subepithelial connective tissue. Note the positive hair follicles and the negative epithelium and surrounding subepithelial tissue. Gomori technique, incubation time 30 min. $\times 25$.

Fig. 4. Margin of a healing wound in the rectum of the rat. On the left, the organizing granulation tissue gives a strongly positive reaction for phosphatase. On the right, the undisturbed mucosa is negative apart from a number of positive blood vessels. Gomori technique, incubation time 30 min. $\times 35$.

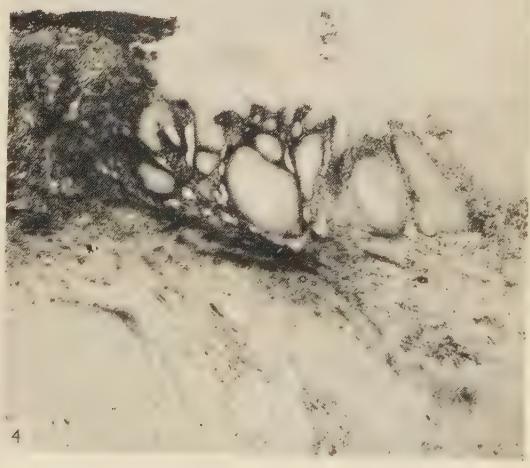
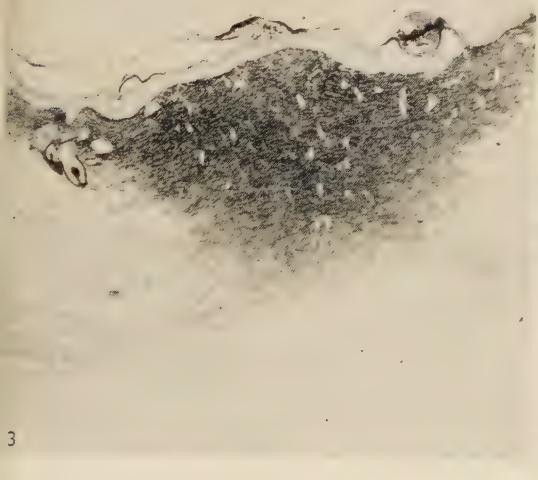
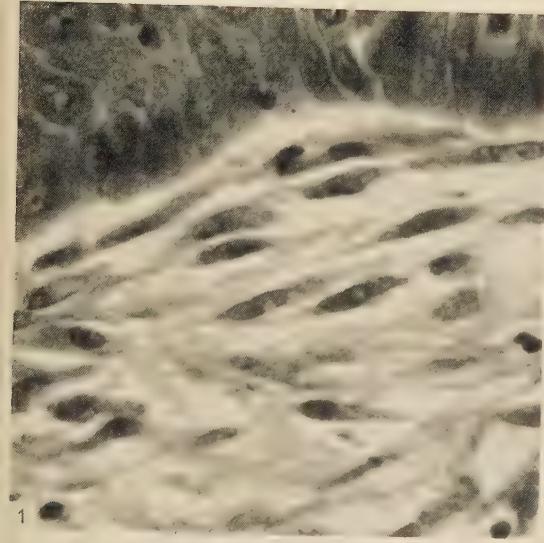
PLATE 2

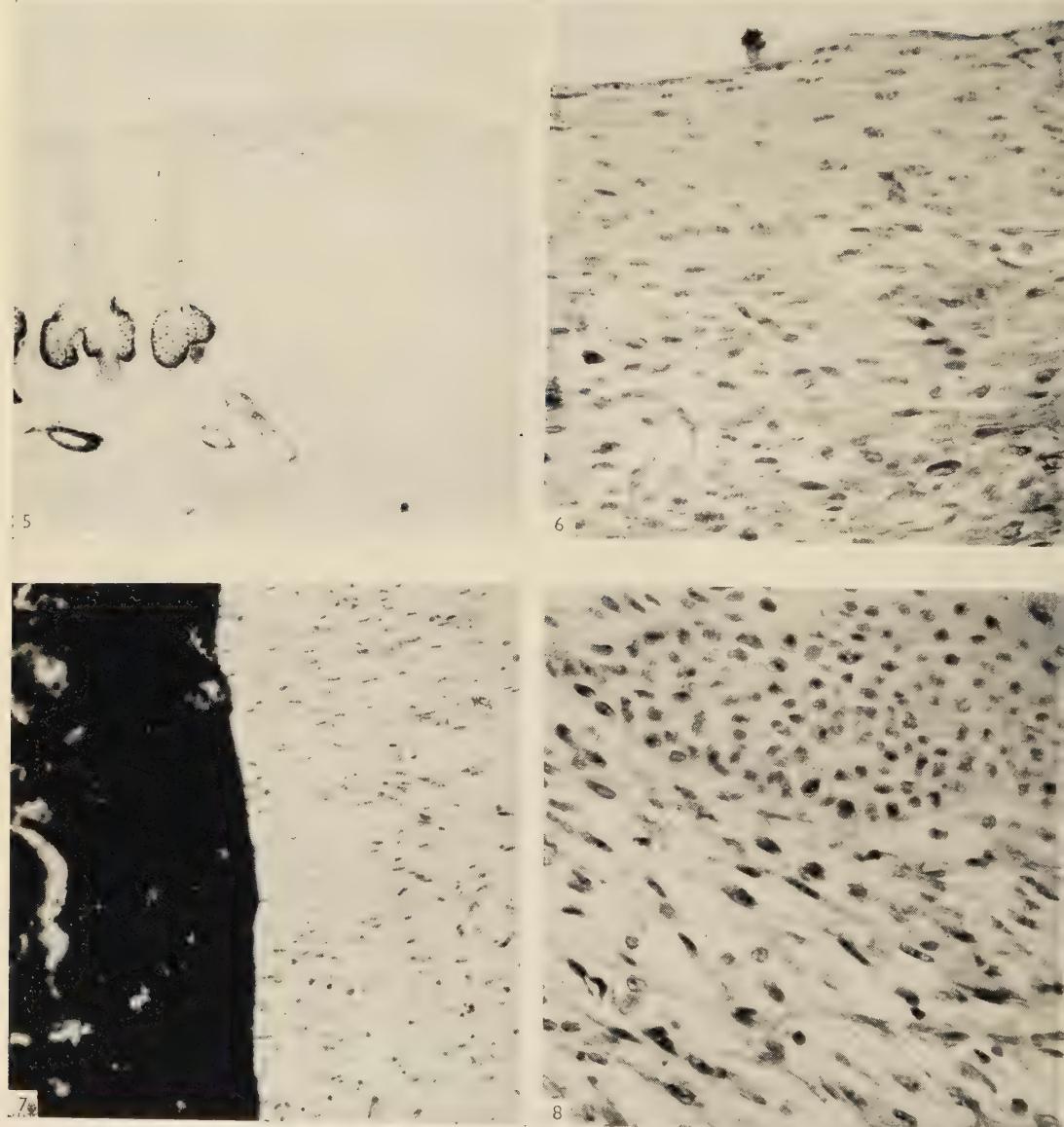
Fig. 5. Margin of a skin wound in the cat. Note the positive reaction for alkaline phosphatase in sebaceous glands in the undisturbed tissue on the left and the negative reaction in the new connective tissue in the wound area on the right. Compare with Pl. 1, fig. 3. Gomori technique, incubation time 3 hr. $\times 66$.

Fig. 6. Section of a wound of rat skin, demonstrating an affinity for alkaline phosphatase. Note that the affinity is shown by all cells, including those of the epithelium, and that the reaction is mainly confined to nuclear membranes and nucleoli. For technique, see text. $\times 345$.

Fig. 7. Section demonstrating diffusion of products of reaction from an overlying section of kidney (left) into inactivated young fibroblasts of a skin wound in the rat. For technique, see text. $\times 260$.

Fig. 8. Section illustrating the affinity of young fibroblasts (below) in a wound of cat skin for calcium phosphate. Note that the epithelial cells (above) show a similar affinity, and that it is mainly possessed by nuclear membranes and nucleoli. For technique, see text. $\times 345$.





THE BEHAVIOUR OF AUTOGRRAFTS OF ILEUM TRANSPLANTED INTO THE URINARY BLADDER IN RABBITS

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INTRODUCTION

For many years surgeons have performed the operation of ileocystoplasty in which a loop of ileum with its blood supply intact is anastomosed to the urinary bladder, part of which has been removed. Siracusano & Manetti (1956), who reviewed the literature, pointed out that the first operation was carried out in dogs in 1888, and in man in 1898. In more recent years the operation has been frequently performed both in animals and in man, and interest has centred mainly on the functioning of the grafted ileum as a substitute for a bladder, most of which has been removed or was diseased. Rubin (1948), using the sigmoid colon in dogs, reported very briefly on the histology of the graft, and stated that its mucous membrane showed no change. Tasker (1953), Davids & Bellwin (1954), Shoemaker (1955), Shoemaker, Tedeschi & Grotzinger (1956), and Siracusano & Manetti (1956) also used dogs, and reported that the ileal mucous membrane showed no change up to 2 years after the operation. Couvelaire (1950, 1951), Cibert, Rolland, Durand & Brandsma (1952), Cibert, (1953) and Pyrah & Raper (1955) carried out the operation of ileocystoplasty in patients, but did not describe the histology of the graft or bladder. In a personal communication, Cibert (1956) stated that a further operation had to be carried out in two of his patients, and in both cases, one 2 years and the other 4 years after the grafting of the ileum to the bladder, a piece of the transplanted ileum was removed, was examined histologically and was found to retain all the characteristics of normal ileum.

This operation of grafting ileum into the urinary bladder affords an opportunity of studying structure in relation to environmental influences, and it was decided to repeat the experiment in rabbits and modify the procedures especially in relation to the blood supply of the graft. As an indication of the functional state of surviving ileum, the alkaline phosphatase activity of the epithelium of the grafted ileum and the bladder was also investigated, together with some features of regenerating transitional epithelium.

MATERIAL AND METHOD

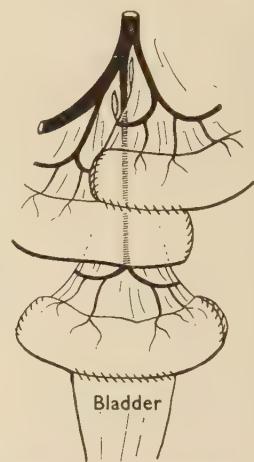
Adult rabbits of mixed stock weighing 1·5-3·0 kg were used. Under intravenous Nembutal anaesthesia, supplemented by ethyl chloride as required, the following experiments were carried out.

(1) *Pedicle grafts.* A loop of ileum about 3 cm. long was isolated with its blood supply intact and continuity of the gut was restored by side-to-side anastomosis. Attempts were made to perform an end-to-end anastomosis, but as a rule this

resulted in obstruction. The bladder was emptied and a piece of its fundus, usually about 1 cm. in diameter, was removed. The ends of the ileal loop were closed, the antimesenteric border of the loop was opened and its edge sutured to the upper cut edge of the bladder. 5/0 U.S.P. silk sutures were used throughout the operation (Text-fig. 1). 20,000 units of penicillin were given intramuscularly immediately after the operation and once daily for the next 2 days. Biopsies were performed on eight rabbits, 7, 12, 20, 30, 40, 50, 100 and 400 days after the operation. Frequently the sites of the side-to-side anastomosis and of the anastomosis of the ileum to the bladder were adherent to surrounding tissues (anterior abdominal wall, abdominal viscera), but the adhesions were easily separated. The ileal graft was identified by the presence of the non-absorbable sutures (Pl. 1, fig. 1) and the part of the bladder containing the graft was removed. Internally the area of the graft was usually easily identifiable (Pl. 1, fig. 2). The 20-, 30-, 40-, 100- and 400-day specimens were divided through the middle of the graft and were pinned on to cork, and one half was fixed in Bouin and the other in chilled acetone. The whole of the 7-, 12-, and 50-day specimens were fixed in Bouin. The specimens fixed in chilled acetone were used for investigating the distribution of alkaline phosphatase in the tissues.

(2) *Patch grafts.* A piece of ileum 2-3 cm long and about 1 cm wide was stitched transversely as a patch into the middle of the posterior wall of the bladder. The continuity of the gut was restored by a side-to-side anastomosis. 5/0 U.S.P. silk sutures were used. The animals were given the same amount of penicillin as in the first experiment. Biopsies were performed in two rabbits after each of the following periods: 10, 20, 35 and 50 days. The site of the graft was identified by the sutures and the grafted area was removed, pinned on to cork and fixed in Bouin. Frequently these specimens had stones attached to the sutures.

(3) *Pedicle grafts with pedicle tied at varying intervals.* The same operation as in the pedicle graft animals was carried out in four rabbits. In these, the pedicle was tied after 21 days, and biopsies were performed 14, 28, 56 and 112 days after the second operation. The bladder wall containing the graft was removed and divided into two; one half was fixed in Bouin and the other in chilled acetone. The latter specimens were used for the detection of alkaline phosphatase. As a result of the observations made on these four rabbits, the pedicle graft operation was performed in three rabbits and the pedicle was tied and cut immediately after the operation. Biopsies were performed after 28, 56 and 112 days. The part of the bladder containing the site of the graft was removed, pinned and fixed in Bouin. In two further rabbits the pedicle was tied after 7 days, and biopsies were performed 35 and 42 days after the second operation. In a series of rabbits, the pedicle graft operation was performed and immediately after the operation and after 1, 2, 3, 4 and 7 days, the pedicle was tied and the vessels of the bladder were injected with Indian ink by means of a cannula inserted into the abdominal aorta just above its bifurcation



Text-fig. 1. Diagram of operation of ileocystoplasty, referred to as a pedicle graft in the text.

to determine the extent to which there were vascular connexions between the bladder wall and the graft.

(4) *Regeneration of mucosa of ileum of pedicle graft.* As a result of some of the peculiar appearances of the transitional epithelium in some of the specimens examined, the pedicle graft operation was carried out in four rabbits, but before anastomosing the ileum to the bladder a small area of the mucous membrane on the mesenteric border of the ileum was removed by scraping with the handle of a scalpel so that there was a denuded area in the middle of the graft. Biopsy was performed 20, 30, 50 and 100 days later. The bladder wall containing the graft was fixed in Bouin.

In all the experiments the Bouin-fixed specimens were sectioned at 7μ , some serially, and stained with haematoxylin and eosin.

(5) *Alkaline phosphatase in lining of bladder and pedicle graft.* The specimens fixed in chilled acetone were sectioned at 7μ and stained by the Gomori method (1952) for alkaline phosphatase.

RESULTS

(1) *Pedicle graft.* In all rabbits the ileum retained its characteristic structure even after 400 days. Usually the ileum formed part of the bladder wall and could be distinguished by its relative thinness. Sometimes the lateral part of the anastomosed loop was adherent to some abdominal structure and formed what was almost a diverticulum of the bladder in its upper lateral part. This is commented on because that part of the ileum which formed part of the bladder wall, although histologically ileum, became stretched so that its normally permanent folds disappeared and even the villi became broader and flatter (Pl. 1, fig. 3). On the other hand, that part of the ileal loop which did not become incorporated into the bladder wall and was not subjected to stretching as the bladder filled, retained the characteristic folds of the mucosa and shape of the villi. The columnar epithelium next to the bladder showed a sheet-like arrangement rather than its usual villous appearance and contained few goblet cells. In the rabbit biopsied after 7 days the edge of the ileal graft was denuded of epithelium. These observations suggested a loss of columnar epithelium which was replaced by a growth of the neighbouring ileal epithelial cells.

In most pedicle grafts the transitional epithelium of the bladder met the columnar epithelium of the ileum in a fairly even line (Pl. 1, fig. 4), but occasionally the transitional epithelium was seen growing over the edge of the columnar epithelium and becoming attached to the tips of neighbouring villi. Sometimes transitional epithelium appeared to be attached to the tip of a villus as an independent 'sprout' (Pl. 1, fig. 5). These latter appearances were seen next to or near the line of anastomosis between the ileum and bladder, and were seen more frequently in the early patch grafts and the rabbits in which the pedicle was tied immediately after the operation. In these rabbits, the transitional epithelium next to the graft showed a large number of mitoses.

(2) *Patch grafts.* In these rabbits there was evidence of degeneration of all the layers of the ileal graft early on, with disappearance of the graft at 50 days and its replacement with fibrous tissue (Pl. 2, fig. 6). In the rabbits biopsied after 10 and 30 days the transitional epithelium was seen growing over the degenerating columnar epithelium.

(3) *Pedicle grafts with pedicles tied at varying intervals.* The bladders of the four rabbits in which the pedicle was tied after 21 days showed normal ileum well integrated into the bladder wall and were similar in appearance to the pedicle grafts described in (1). The bladders of the three animals in which the pedicle was tied immediately after the operation showed disappearance of most of the ileum with the survival of small areas of the graft. After 56 days it was estimated that the original ileal graft, measuring about 3 cm. long and 2 cm. wide, was represented by discontinuous pieces of ileum whose total length was about 0·8 cm. and width about 1 cm. Most of the ileal graft was replaced by fibrous tissue and the transitional epithelium was seen growing over the columnar epithelium (Pl. 2, fig. 7). After 112 days pieces of ileum still survived, but nowhere in the bladder did the grafted ileum form part of the wall in the same way as the pedicle graft; the ileal mucous membrane appeared to be in two parts each adjacent to the sutured edge. One part, of very varying width (0·5–5 mm.) and 6 mm. long was completely buried by transitional epithelium and connective tissue, and the other, about 6 mm. wide and 5 mm. long, formed part of the lining of the bladder and extended into the wall. The transitional epithelium was obviously growing close to this, resulting in the bizarre appearances seen in Pl. 2, fig. 8.

In the two rabbits in which the pedicle was tied after 7 days, the ileal graft retained its characteristic features after 35 and 42 days and formed part of the bladder wall. In the rabbit in which after 7 days the pedicle was tied and Indian ink was injected into the abdominal aorta, it was observed that the ink passed from the vessels of the bladder into the vessels of the graft and pedicle. In the rabbits in which Indian ink was injected immediately after the operation and after 1 day, ink particles were seen lying in the tissue spaces of the graft, but not in its vessels. After 2, 3 and 4 days, the ink was in the tissue spaces and vessels of the graft (Pl. 2, fig. 9).

(4) *Regeneration of mucosa of ileum of pedicle grafts.* In the four rabbits of this series the mucosa of the ileal graft was replaced by columnar epithelium growing from the surrounding ileal mucosa (Pl. 2, fig. 10). In view of this and in order to explain the appearance of independent 'sprouts' of transitional epithelium attached to the tips of villi, serial sections of many of the specimens in all the experiments were re-examined. These showed that epithelium, apparently separated from the main lining of the bladder, was really continuous at some point with the rest of the transitional epithelium. The microscopic appearances seen in Pl. 1, fig. 5 (left) are due to an irregular edge of transitional epithelium growing over part of the ileal graft, towards the degenerating tips of nearby villi.

(5) *Alkaline phosphatase in lining of bladder and pedicle graft.* Normally this enzyme is found in the deeper layers of the mucosa of the bladder and in the free edge of the columnar cells of the ileum. Up to 30 days there was no alteration in this distribution. In the pedicle grafts after 30 days, although the bladder mucosa showed no change, the ileal mucosa usually had areas with either no phosphatase or a decrease. This loss did not always occur or might be restored, since the 100-day pedicle graft showed a normal distribution of phosphatase in the ileal mucosa.

DISCUSSION

The first part of this investigation confirmed the results found by other workers, namely that a graft of ileum (or large bowel) to the bladder, complete with its blood supply, forms part of the bladder wall and histologically retains its own structure. Many workers have investigated the functions of the grafted ileum (Covelo, 1955; Marucci, 1955; Marucci, Shoemaker, Wase, Strauss & Geyer, 1954; Pyrah, Care, Reed & Parsons, 1955) and they have shown that K, Ca, P, urea and chloride are all absorbed from the urine and that mucus continues to be secreted.

No references in previous work have been made to the changes in the folds and villi of the graft. It is usually said that filling of the intestine does not iron out its folds (Ham, 1957), but it appears that the ileum undergoes some change when grafted to the bladder, and distension of that organ leads to a flattening not only of the folds but of the villi as well. This would appear to be an important structural change due to the change in environment. Another change, the significance of which it is difficult to assess, is the decrease or disappearance of the alkaline phosphatase from the epithelial cells of the mucosa.

Other workers have not commented on the appearances of the adjacent edges of the bladder and ileum. In the present experiments the edges sometimes met in a straight line, but frequently the transitional epithelium appeared to be growing over the ileum to a greater or less extent. Near the suture line there was some degeneration of either the transitional epithelium of the bladder or the columnar epithelium of the ileum or both and to some extent both epithelia could regenerate to cover the defect. The columnar epithelium covered part of the denuded surface of the ileum but never appeared to grow over the bladder surface. On the other hand, the transitional epithelium not only lined the denuded area of the bladder but frequently grew over the ileum towards the tips of neighbouring villi to which it became attached if their epithelium had disappeared. The absence of covering cells can act as a stimulus to the epithelia and in the environment of the bladder the tendency is for the transitional epithelium to grow much more than the columnar epithelium.

Epithelial regeneration in the middle of the ileal graft on the other hand was entirely by columnar epithelium. It is interesting to compare the appearances of the regenerated epithelium with those described by McMinn & Mitchell (1954) in their experiments on the cat in which they produced artificial lesions of the mucosa of the small intestine. They found that the crypts and villi were reconstituted after 100 days. In the present experiments the epithelium covering the denuded area remained as an even sheet of columnar epithelium after the same period, and no villi or crypts were present. In both experiments cyst-like spaces were seen deep to the epithelium. The absence of crypts and villi in the regenerated lining of the ileal graft was almost certainly due to the fact that it formed part of the bladder and was repeatedly distended by the urine in that organ.

The persistence of some part of the pedicle grafts deprived of their blood supply immediately after the operation can be explained by the findings following injection of Indian ink immediately after the operation. With the tying of the pedicle the ileum sewn on to the bladder could be seen to change from a healthy pink to the typical purple of the gut deprived of its blood supply and it was expected that the

graft would rapidly necrose and perforate. All three rabbits lived, and although a great deal of the ileum degenerated and was replaced by or became fibrous tissue, some of its muscle and columnar epithelium survived even after 112 days. One can only assume that serum from the bladder oozes across the cut edge in sufficient quantity to prevent necrosis of the graft. It is difficult to assess how permanent were the remains of the graft but much of the epithelium that survived was healthy. On the other hand, the transitional epithelium near the graft appeared to be growing over the remaining columnar epithelium.

The rapidity with which the vessels of the graft connected with the vessels of the bladder wall was also striking. Two days are a very short period for vascular connections to be established and the short time required may be related in some way to the fact that Indian ink was present in the graft in those animals in which the pedicle was tied immediately after the operation. The length of time can be compared with the 4 days required for the vascularization of autografts of skin (Medawar, 1944).

Finally, one may comment on the tendency for transitional epithelium to regenerate, spread and line any non-epithelialized part of the inside of the bladder. This process was described by Baret, De Muth, Murphy & Muir (1953), who replaced the bladder wall with fascia from the rectus sheath, and by Grotzinger, Shoemaker, Marucci, Ulin & Martin (1954) who used an inverted seromuscular graft in which the peritoneum formed the lining of the graft and was continuous with the lining of the bladder. Bohne, Osborn & Hettle (1955) removed the bladder, replaced it with a plastic mould to which the ureters and urethra were joined, and after 15 weeks found round the mould a new bladder consisting of granulation tissue lined with transitional epithelium. Sanders & Schein (1956) replaced the ureter or bladder with different structures, and found that transitional epithelium lined non-epithelialized tissues, e.g. arterial grafts and abdominal wall grafts, with the serous surface forming the lumen. McMinn & Johnson (1955) removed part of the lining of the normal bladder and found that a denuded area of 1 cm.² was covered by transitional epithelium by the end of the second week. Similarly, in the present investigation, transitional epithelium usually covered a graft which lost its epithelial lining and even grew over remaining columnar epithelium if the tips of neighbouring villi were denuded. On the other hand, the intact epithelium of a graft persisted whether it was the epidermis (Draper & Stark, 1956), the columnar epithelium of the small intestine, both in the present investigations and the others referred to in the introduction, or that of the uterine tube (Sanders & Schein, 1956). One wonders what stimulates the growth of the transitional epithelium and why the stimulus is only present or effective in the absence of an epithelial lining.

SUMMARY

1. Ileocystoplasty, i.e. the grafting of a loop of ileum with its blood supply intact to the bladder, has been performed in rabbits (pedicle grafts).
2. As in other experimental animals and in man, the ileum retained its basic structure and its own columnar epithelium up to 400 days after the operation. The main changes in the ileum were the flattening and broadening of the villi, the

disappearance of the normal folds and the reduction or disappearance of the alkaline phosphatase of the columnar epithelium.

3. A free patch graft of ileum into the bladder was replaced after 50 days by fibrous tissue and lost its epithelial lining which was replaced by transitional epithelium.

4. If after ileocystoplasty the pedicle were tied at the end of 7 days, the ileum was seen to retain its basic structure 35 and 42 days after the tying. If the pedicle were tied immediately after the operation, the ileum showed considerable changes after 28, 56 and 112 days but some of the columnar epithelium survived. By Indian ink injection it was shown that serum oozes from the cut edge of the bladder into the graft immediately after the operation and that the graft acquired a good vascular connexion with the bladder after 2 days.

5. In a series of pedicle grafts, the ileal mucous membrane was removed from the middle of the graft and it was found that the denuded area was covered by columnar epithelium after 20 days.

6. The tendency for transitional epithelium to grow and line any non-epithelialized part of the bladder wall or its substitute is commented on.

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EXPLANATION OF PLATES

(All sections were cut at 7μ and stained H. & E., except that of fig. 9 which was cut at 20μ and unstained.)

PLATE 1

Fig. 1. The external appearance of a pedicle graft 40 days after operation.

Fig. 2. The internal appearance of the same pedicle graft as fig. 1.

Fig. 3. Section of the middle of a 40-day pedicle graft to show the flattening and broadening of the villi and the disappearance of the usual folds.

Fig. 4. Section of the junction of the transitional and columnar epithelium in a 50-day pedicle graft.

Fig. 5. The transitional epithelium of a 40-day pedicle graft growing over the adjacent columnar epithelium with an apparently independent 'sprout' of transitional epithelium attached to the tip of a villus on the left.

PLATE 2

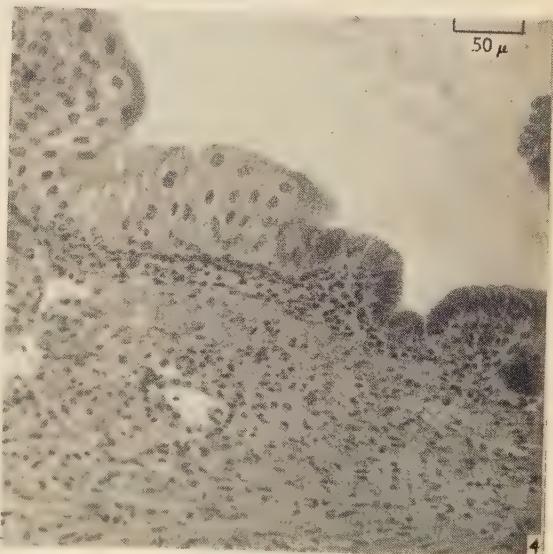
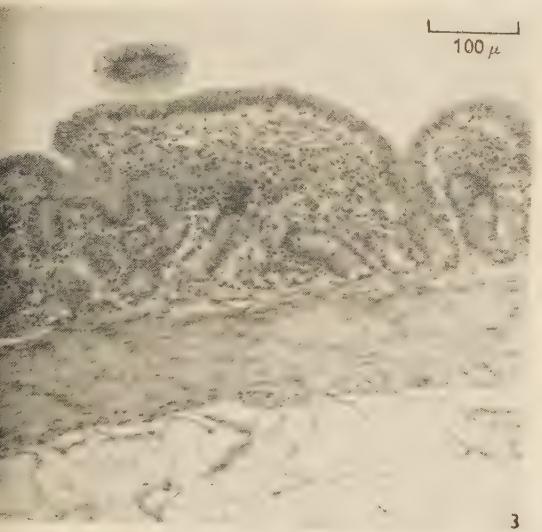
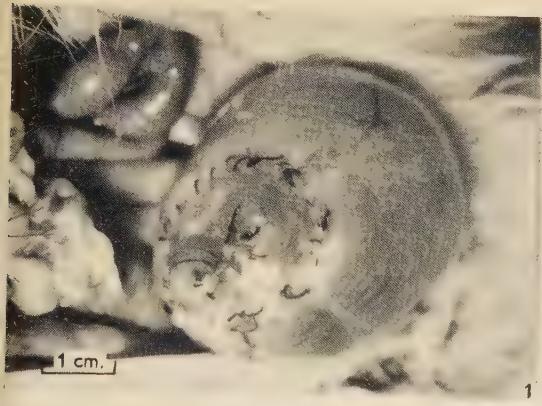
Fig. 6. Replacement of a patch graft by fibrous tissue lined with transitional epithelium after 50 days. The bladder muscle can be easily seen on each side of the fibrous tissue.

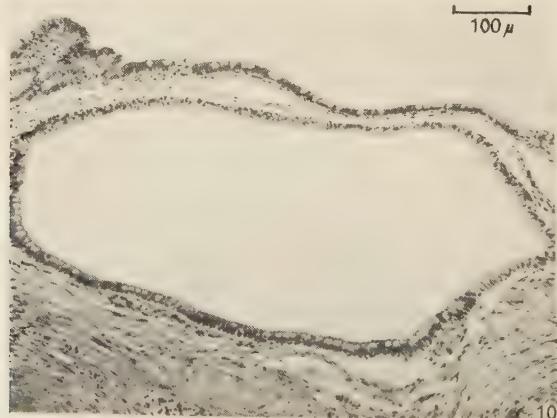
Fig. 7. Low-power microphotograph to show the amount of columnar epithelium persisting after 56 days in a pedicle graft in which the pedicle was tied immediately after the operation.

Fig. 8. The mixture of transitional and columnar epithelium seen near the suture line after 112 days in a pedicle graft in which the pedicle was tied immediately after the operation.

Fig. 9. Indian ink particles in the tissue spaces and vessels of a 2-day pedicle graft. The pedicle was clamped and the Indian ink injected into the abdominal aorta just above its bifurcation.

Fig. 10. The regenerated mucosa in the middle of a pedicle graft 100 days after the operation in which some of the mucosa had been removed.





THE STRUCTURES OF FAST AND SLOW MUSCLE FIBRES IN THE FROG

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INTRODUCTION

In the last decade the physiology of vertebrate fast and slow striated muscle fibres (see, for example, Kuffler & Gerard, 1947; Katz, 1949; Kuffler & Vaughan Williams, 1953) has been given much attention, but, with the exception of Krüger (1952) and his school, their comparative histology has been neglected. Krüger maintains that in cross-section, fast fibres have a fibrillar pattern ('fibrillenstruktur') and slow fibres an areal pattern ('felderstruktur') (see Pl. 1, fig. 1). More recently, Krüger & Günther (1956*a, b*) state that these structures are related to differences in the sarcoplasmic reticulum of the two sorts of fibres seen when impregnated with gold chloride. After denervation mammalian fast fibres give the typical slow-fibre ACh response and, in keeping, their fibrillar pattern changes to the areal pattern.

Electron-microscope studies have been made of the sarcoplasmic reticulum (see Bennett & Porter, 1953; Porter & Palade, 1957), but so far there appears to have been no report of structural differences relating to the two sorts of fibres, although H. E. Huxley (unpublished) has observed two types in frog muscle which might be interpreted in this way.

Krüger's theory has been criticized chiefly because he has shown certain mammalian muscles to contain extrafusal fibres with areal pattern, which on physiological examination failed to show slow-fibre characteristics (see Kuffler & Vaughan Williams, 1953; Granit, 1955). Also it has been suggested that the different patterns are merely fixation artefacts (see Häggqvist, 1956).

No published studies appear to exist of the occurrence of fibrillar pattern and areal pattern in the muscles of the frog *Rana temporaria*. It is therefore of interest to investigate the occurrence, distribution and size of these types in (*a*) a muscle known from physiological and other evidence to contain both types of fibres, and (*b*) a muscle thought to contain no slow fibres.

METHODS

The muscles chosen from *Rana temporaria* were the long extensor muscle of the fourth toe (see Gray, 1957) and the sartorius. The former from physiological evidence is known to contain both fast and slow fibres (Katz, 1949; Kuffler & Vaughan Williams, 1953), whilst no slow fibres have yet been detected in the latter (Kuffler & Gerard, 1947). Also both muscles are strap muscles, having parallel fibres. This is important since each fibre must be sectioned exactly at right angles to the long axis, otherwise the difference between fibrillar pattern and areal pattern is obscured. Also good fixation and minimal shrinkage are essential to demonstrate these two types of structures. These conditions were achieved by exposing the muscles after

pithing, and fixing *in situ* with Bouin's fluid for 5–10 min. Afterwards the central third was carved out together with parts of the underlying muscles and placed in the fixative for 24 hr. The muscles were washed in tap water for 30 min. and then embedded in carbowax (see Armstrong, Richardson & Young, 1956). Carbowax was used because (a) it caused less shrinkage of this material than paraffin wax, and (b) it sets slowly and does not need plunging into cold water, thus permitting ample time to orientate the muscle in the block to produce an accurate right-angle plane of cross-section. Cubical containers were used in embedding to aid orientation. The disadvantage of carbowax is that it is readily soluble in almost every known liquid (mercury being an exception), so that the sections cannot be floated to flatten them. However, the small size of these muscles allowed 10 μ sections to be cut, which did not require flattening. In this case they were pressed firmly with filter-paper (without rubbing) on to albuminized slides and then put into absolute alcohol. The latter precipitates the albumen and sticks the section to the slide. Also the alcohol dissolves the carbowax away slowly (in contrast to water) so that there is less risk of the sections becoming detached from the slide. The sections were then hydrated, stained with haematoxylin and eosin and finally mounted in Canada balsam.

RESULTS

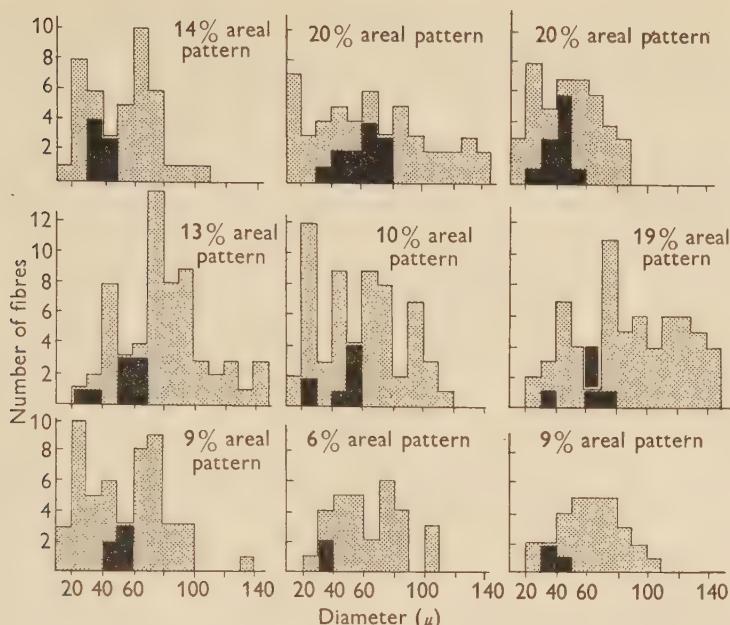
Long extensor of fourth toe

Successful sections were prepared from nine muscles of extensor longus digit IV. Each was seen to contain fibres with either fibrillar pattern or with areal pattern (see Pl. 1, fig. 1) and the latter were not confined to any particular region of the muscle. They occurred in the superficial layer of fibres or amongst the deeper ones, singly or in contiguous groups of two or three. The relative numbers of each and their diameters are expressed graphically in Text-fig. 1. Since the fibres are not circular in cross-section, their greatest diameter was measured. Fibres below 10 μ (these are nearly all intrafusal) were ignored since they were found too small to distinguish in them fibrillar pattern from areal pattern.

In this muscle the results show a clear similarity of constitution between the muscles of different individuals. Each contained only a small proportion of areal-patterned fibres (6–20 %), whose diameters never exceeded 80 μ and, when all were plotted together, showed a peak distribution from 40 to 50 μ . Those with fibrillar pattern tended to be larger, with diameters sometimes reaching 150 μ and a peak distribution from 60 to 70 μ .

Sartorius

Examination of eleven sartorius muscles in this way showed a complete absence of areal-patterned fibres in each. By contrast the deep muscles adjacent to sartorius showed clearly both areal- and fibrillar-patterned fibres (Pl. 1, fig. 2). In this photograph only a small region of the sartorius is shown since the muscle has about 600 fibres and high magnification is necessary to show clearly the different patterns of the two types of fibre.



Text-fig. 1. The size and distribution of fibres with areal pattern and fibrillar pattern seen in cross-sections of nine long extensor muscles of the IVth toe of *Rana temporaria*. ■, areal pattern; ▨, fibrillar pattern.

DISCUSSION

Slow muscle fibres have characteristic 'grape' end-plates, which are morphologically distinct from the end-plates of fast fibres (Günther, 1949; Couteaux, 1952; Gray, 1957). Measurements made by Gray (1957) also on the extensor longus digit IV have shown fibres with grape end-plates having diameters varying from 10 to 80 μ with a peak at 40–50 μ , and fibres with twitch end-plates having diameters varying from 30 to 120 μ with a peak at 60–70 μ . Comparison of size distribution thus supports the view that fibres with areal pattern are slow fibres.

The small proportion of areal-patterned fibres also agrees with the evidence that when this muscle is investigated physiologically, though both types of fibre are encountered (Kuffler & Gerard, 1947; Katz, 1949; Kuffler & Vaughan Williams, 1958), fast fibres are encountered much more frequently than the slow ones. Obviously if both were met with equal frequency, or slow more often than fast, the theory would be suspect.

Finally, the complete absence of areal-patterned fibres in eleven sartorius muscles investigated agrees with the evidence of Kuffler & Gerard (1947) that no slow fibres could be detected in sartorius by physiological means. Also, Krüger (1952) has reported the absence of areal-patterned fibres in the sartorius of another species (*Rana esculenta*).

More direct proof would be obtained if individual fibres known to be fast or slow could be isolated and sectioned separately and their pattern ascertained. The author found it possible to isolate fibres with grape end-plates from the fast fibres (methylene blue stain, Gray, 1957) and to section them separately. However, this stain

requires a special fixative (ammonium molybdate or similar fluid), which unfortunately is such a poor fixative of the muscle fibres that their cross-sectional pattern, whether fibrillar or areal, could not be determined.

SUMMARY

1. Nine ext. long. dig. IV muscles of frogs (*Rana temporaria*) were seen in cross-section to contain both fibrillar-patterned and areal-patterned fibres.
2. There were fewer fibres with areal pattern (6–20 %) and they tended to have a smaller diameter than those with fibrillar pattern.
3. Eleven sartorius muscles all showed a complete absence of areal-patterned fibres.
4. These facts support the theory that when fixed and sectioned by a suitable method, fast fibres show a fibrillar pattern in cross-section and slow fibres show an areal pattern.

I am indebted to Prof. J. Z. Young for advice, to my wife for help with German translations and to Mr R. H. Ansell for technical assistance.

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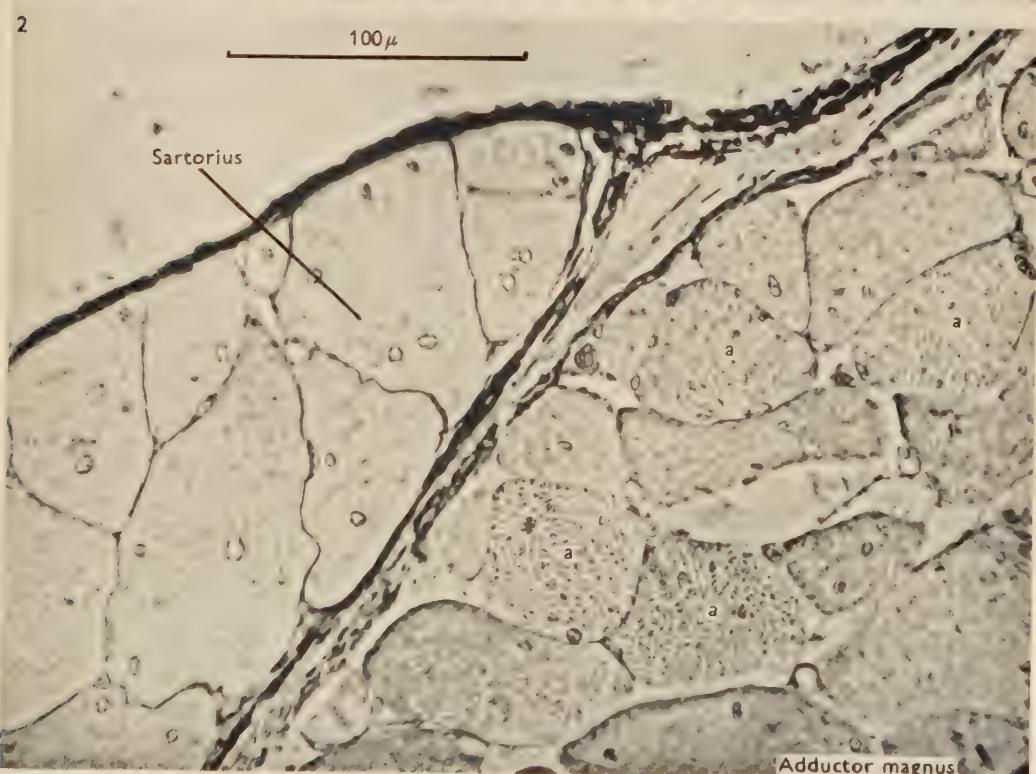
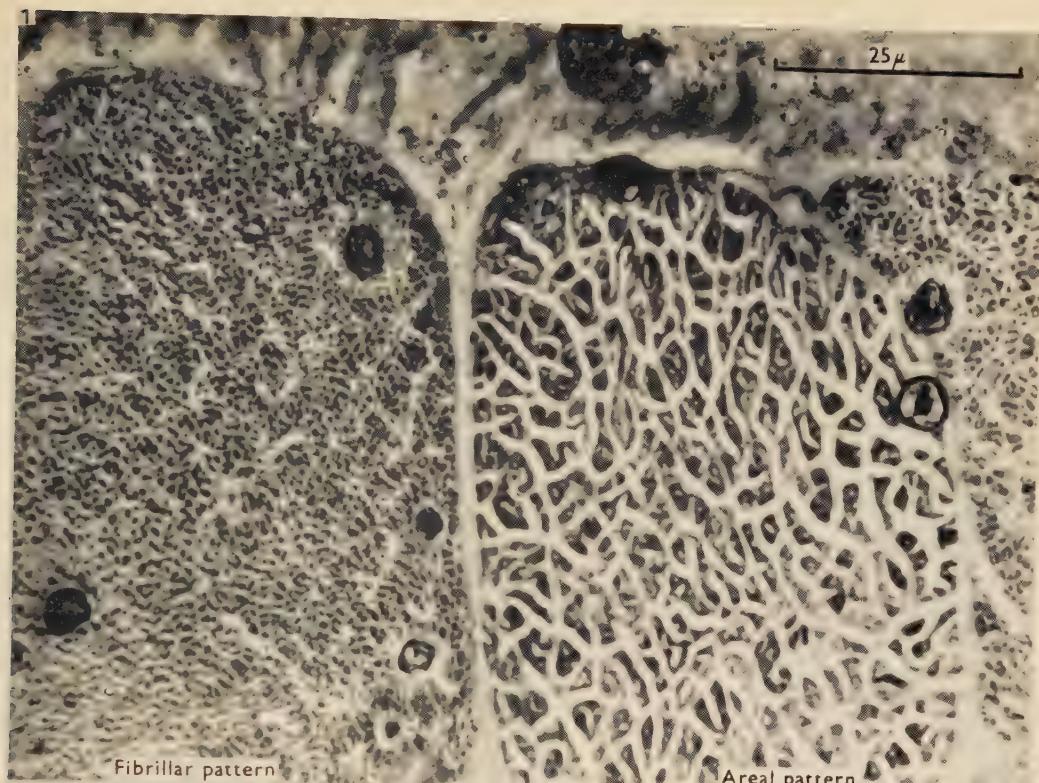
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EXPLANATION OF PLATE

Fig. 1. The two types of fibre pattern seen in cross-section of the extensor longus digit IV of *Rana temporaria* (6 μ section, haematoxylin and eosin stain, phase contrast).

Fig. 2. Fibres with areal pattern (a) are absent in sartorius, but present in adjacent muscles.

Part of adductor magnus deep to sartorius is shown here. Since sartorius contains about 600 fibres only a very small portion can be shown at this magnification. Method as fig. 1.



STUDIES ON HAIR GROWTH IN THE RABBIT

By H. J. WHITELEY

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In the study of factors that might be responsible for controlling the mechanism of hair growth use has been made of the cyclical wave of hair replacement that passes over the trunk of the rat and rabbit (Durward & Rudall, 1949; Whiteley & Ghadially, 1954). Durward & Rudall (1949) showed that in the rat this wave of growth normally passed from the venter to the dorsum and that when a square of skin on the flank was rotated through 180° the direction of the wave of growth in this skin was changed, passing from dorsum to venter. They therefore concluded that the direction of the wave was inherent in the skin. More recently, Ghadially (1957) has demonstrated a similar mechanism in the rabbit as the transposition of skin flaps on the trunk modified the wave of growth, and that the wave of growth changed its direction in correspondence with the reorientation of the skin. These two groups of experiments suggest that the wave of hair growth is the result of some inherent property of the skin. However, in both these transposition experiments the skin flaps were not separated from their original nervous and blood supply and therefore the possibility remains that the activity of the skin might be the result of nervous or vascular mechanisms, although Durward & Rudall (1949) showed that denervation of the skin did not greatly modify the wave of hair replacement in the otherwise normal rat.

It is well known that the hair growth cycle can be initiated by plucking out the hair, and it has been stated that when the plucked area has regrown the follicles are thereafter out of phase with the normal growth wave (Chase, 1954). This supports the idea that the mechanism for control of hair growth is due to intrinsic factors in the skin and that there is no systemic regulating mechanism to re-establish the normal cyclical pattern of growth.

It was decided to investigate both these problems in the rabbit: first, to see whether the intrinsic activity was maintained in full thickness transplants separated from their original nervous and vascular supply; and secondly, to determine whether the normal pattern of growth is ever re-established once an area of skin is put out of phase. It was found that the skin transplants retained their original intrinsic activity and were not modified by the activity of the surrounding tissue, and that the normal pattern of growth was ultimately re-established in all the animals observed.

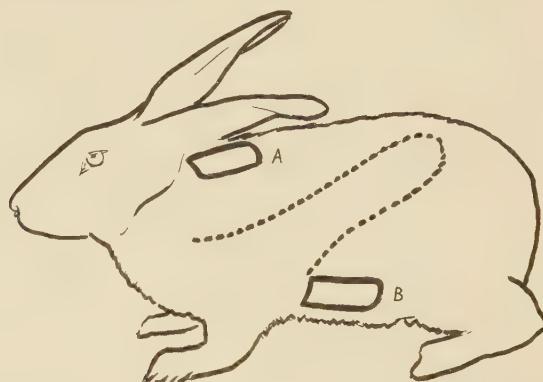
METHODS

The transplants were made using a tube pedicle graft. Under the ether anaesthesia an incision was made in the flank of the rabbit (Text-fig. 1) through the panniculus carnosus. The cut edges of the skin were joined to form a skin tube and the free end of the tube was sewn into an incision made either in the back of the neck or on the belly. In all, eight animals were used; in five the graft of skin from the dorsum was put into the back of the neck and in three into the belly. It was important while

making the incision for the tube to see that the large vessels supplying the skin were not damaged. The pedicle was left for 6–8 weeks to ensure that an adequate blood supply was established and then the pedicle was divided. The full thickness graft survived in all eight animals. The animals were clipped and photographed at regular intervals and observed for periods of up to 3 years.

Four chinchilla rabbits had the right flank plucked and one had a narrow band of hair encircling the trunk plucked. These five animals were clipped and photographed at regular intervals and observed for periods up to 3 years.

All animals were housed in individual cages and were fed the usual bran and oat mash with supplements of greens.



Text-fig. 1. Showing the line of the incision for making the pedicle graft. The free end of the graft consisting of skin from the dorsum was inserted into the back of the neck (A) in five rabbits and into the belly (B) in three rabbits.

RESULTS

A. Skin transplants

In all the rabbits that had full thickness skin transplants the transplanted skin behaved as if it had not been removed from its original site. When the dorsal hair growth started at the commencement of the cyclical process of hair replacement the transplanted skin which was from the dorsum became active, whether it was in the belly or the neck (Pl. 1, figs. 1, 2). This was observed in all successive cycles until the animals were killed (Pl. 1, figs. 3, 4).

It is interesting to note that the growth in the transplanted skin did not extend into the surrounding skin nor did growth in the skin surrounding the transplant affect the transplant although the nerve and vascular supply must have been that of the surrounding skin. These results confirm the work of Durward & Rudall (1949) and Ghadially (1957), that the skin has an intrinsic pattern of growth which is not controlled by nervous or vascular factors.

B. Hair-plucking experiments

In the four rabbits that had the right flank plucked the normal pattern of growth was ultimately re-established. Three of the animals showed a gradual return to the bilateral symmetrical pattern over the period of 3 years (Pl. 2, figs. 7–11), but in

one animal the normal pattern of growth was suddenly established at the next but one growth period (Pl. 1, figs. 5, 6).

In the one animal that had a narrow band of hair plucked from the trunk the normal symmetrical pattern of growth was established in 2 years (Pl. 2, figs. 12, 13).

DISCUSSION

The results reported on the whole thickness skin grafts show that there is some inherent factor or mechanism in the skin, independent of nervous or vascular control, concerned in the growth and replacement of hair, for when a wave of growth starts on the dorsum, growth occurs simultaneously in the transplanted skin, whether it is on the belly or the back of the neck. This is in agreement with the findings of Durward & Rudall (1949) and Ghadially (1957) in their studies on the hair growth cycle in the rat and rabbit, although in their work the possibility of some control through the nervous system was not completely excluded.

This cyclical wave of growth is known to occur annually or biannually in the rabbit (Whiteley & Ghadially, 1954) and in the arctic hare it is definitely seasonal in its incidence, being associated with the seasonal alteration in the period of daylight (Lyman, 1942). It seems probable that this seasonal cycle is initiated by changing hormonal factors acting selectively on areas of skin that are inherently more susceptible than the rest of the skin, in the rabbit this area being the skin of the dorsum. It is known that hormones can affect the hair growth cycle, for cortisone will inhibit the mechanism responsible for the initiation of the hair growth cycle that follows plucking of the hair in the rat and rabbit (Whiteley, 1958), and that adrenalectomy initiates a wave of hair growth in the rat (Montagna, 1956; Whiteley, 1958).

It was thought that this pattern of hair replacement could be put permanently out of phase by plucking the hair from an area of skin and so artificially stimulating the hair to grow in any selected portion of the skin (Chase, 1954). This naturally emphasized the importance of the local mechanism rather than possible systemic factors. However, the experiments reported here, where areas on the flank were put out of phase by plucking out the hair, show that after a varying period of time the normal symmetrical pattern of hair growth was re-established, presumably due to the action of systemic factors. This was strikingly shown in a rabbit where the symmetrical pattern was re-established at the next-but-one wave of hair replacement. The importance of systemic factors is also observed occasionally, for the pattern of growth can be reversed by thyroidectomy and hypophysectomy (Dieke, 1948) and in the arctic hare the spring moult is from the dorsum to the venter and the autumn moult from the venter to the dorsum (Lyman, 1942).

Kleitman (1949), in an extensive review of biological rhythms and cycles, states that: 'Cycles, while essentially inherent, can be modified by internal (nervous, endocrine) and external influences in the latter case . . . , becoming synchronized with an external periodicity.' It seems that the cycle of hair growth in the rabbit fits into this definition for the experiments on skin transplantation indicate that there is an inherent mechanism in the skin, and the hair-plucking experiments show that there is some internal modifying influence. Finally, it is clearly seen in the arctic hare that the cycle of the moult is synchronized with an external periodicity.

SUMMARY

Full thickness skin transplants were made using pedicle grafts, in which skin from the dorsum was transplanted into the back of the neck in five rabbits and into the skin of the belly in three rabbits. After the grafts had taken the pedicle was divided, thus separating the transplanted skin from its original nervous and vascular supply. The animals were observed for periods up to 3 years. When hair growth occurred on the dorsum there was simultaneous growth in the transplanted skin in all animals.

The normal symmetrical pattern of hair growth was put out of phase by artificially stimulating hair growth by plucking out hair from the right flank in four rabbits and in a band encircling the trunk in one rabbit. In all animals the normal symmetrical pattern was ultimately re-established.

These results are discussed and it is concluded that although there is some intrinsic mechanism in the skin, independent of nervous or vascular control, concerned in the hair growth cycle, this mechanism is subject to internal modifying influences which are hormonal in nature.

I am indebted to Mr J. P. Napper for the photographs of the skin specimens and to Miss J. Williams for the drawing.

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EXPLANATION OF PLATES

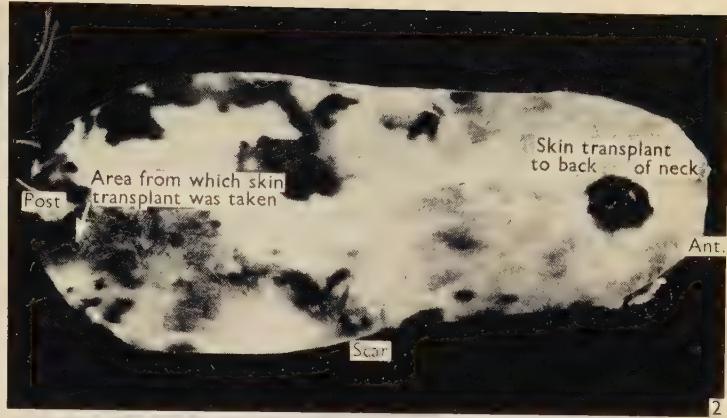
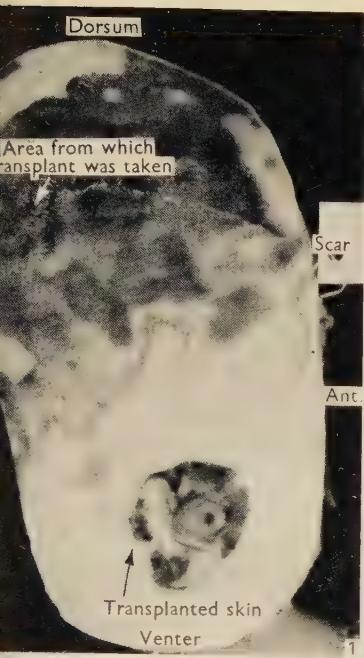
(In all illustrations the letter *H* indicates the head)

PLATE I

Fig. 1. RB/52/53. Specimen of skin from the flank of the rabbit, killed January 1956, that had a transplant into the belly in July 1953, showing simultaneous growth of hair in the dorsal skin and in the transplant. The appearances in the first and second year are shown in figs. 3 and 4.

Fig. 2. RB/35/53. Specimen of skin from the back of the rabbit, killed in January 1956, that had a transplant into the back of the neck in May 1953, showing simultaneous growth of hair in the transplanted skin and in the dorsal skin.

Fig. 3. RB/52/53. Flank (December 1953) showing simultaneous growth of hair on the upper part of the flank and in the transplant in the first year after transplantation.



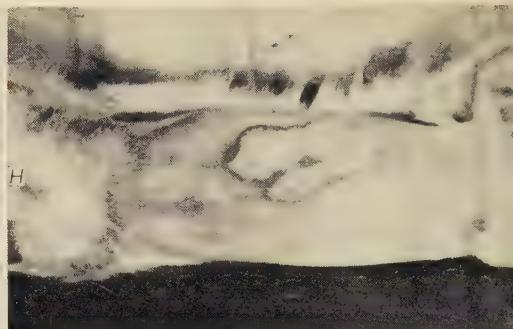
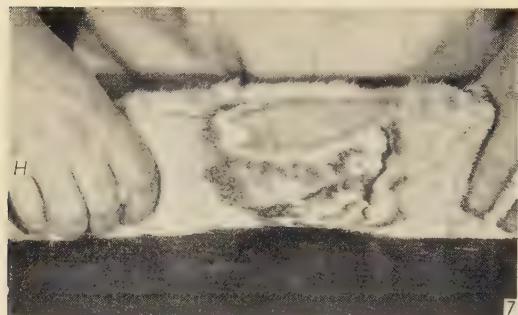


Fig. 4. RB/52/53. Flank (January 1955) showing simultaneous growth of hair in the dorsal skin and in the transplant in the second year after transplantation. The pattern of growth is very similar to that in figs. 1 and 3, and there has been no spread of growth from the transplant into the surrounding quiescent skin.

Fig. 5. RB/13/54. Showing asymmetrical growth of hair after plucking the right flank (January 1954). The next cycle was asymmetrical but symmetrical growth was suddenly established in December 1954 and was maintained in subsequent cycles (fig. 6).

Fig. 6. RB/13/54. Dorsum of rabbit showing symmetrical pattern of growth, November 1955.

PLATE 2

Fig. 7. RB/78/53. Dorsum of rabbit showing asymmetrical growth on the left flank, February 1954. The right flank was plucked, November 1953, and the skin put out of phase.

Fig. 8. RB/78/53. Dorsum of rabbit showing asymmetrical growth on the right flank, March 1954, growth on the left flank having been completed.

Fig. 9. RB/78/53. Dorsum of rabbit showing asymmetrical growth on the left flank, May 1954, of almost identical pattern to that in fig. 7.

Fig. 10. RB/78/53. Dorsum of rabbit with persistence of the asymmetrical pattern of growth, showing hair growth on the right flank, December 1954.

Fig. 11. RB/78/53. Dorsum of rabbit, November 1955. There has been almost complete re-establishment of the symmetrical pattern of growth, and in the next cycle full symmetrical growth occurred.

Fig. 12. RB/54/53. Dorsum of rabbit showing regrowth of hair after a band of hair encircling the trunk was plucked in October 1953.

Fig. 13. RB/54/53. Showing the re-establishment of the symmetrical pattern of growth after 2 years. Dorsum, October 1955.

ACROCEPHALO-OLIGODACTYLISM IN A WILD CHIMPANZEE

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INTRODUCTION

That in man deformities of the head, hands and feet not infrequently occur in combination is evident from the large number of such reports in the medical literature. The name 'acrocephalo-syndactylism' was introduced in 1906 by the French paediatrician Apert for the joint occurrence of an abnormally high head and malformed digits which he had regarded as a clinical entity. Of the numerous later relevant publications the one by Park & Powers (1920) is specially noteworthy, not only because these authors collected a total of twenty-nine cases of acrocephalo-syndactylism, but also since they showed that different deformations of the cranial vault can be associated with various kinds of anomalies in the hands and feet, indicating thereby simultaneous disturbances early in development. The case to be described here belongs clearly in the same general teratological category inasmuch as it shows the abnormally high cranial vault, called acrocephaly, combined with an extreme, symmetrical loss in digital development, properly called oligodactylism. That this syndrome can occur even in non-human primates is not really surprising, but since it has not before been found among the latter it deserves to be recorded. As far as the writer can ascertain, there exists no report on real acrocephaly in any monkey or ape. In contrast to this, digital malformations of practically all the kinds known in man have been described for numerous monkeys and especially apes, among which they can be even more common in some species than in man (Schultz, 1944, 1956).

The chimpanzee to be discussed was obtained in August 1957 by Mr Phillip J. Carroll of Yaounde, French Cameroon, who kindly sent the preserved body to the writer together with the following information: 'I send you an embalmed body of a rare chimpanzee which I believe is a new species, he weighs 6 lb., has full second set of teeth, has no thumbs and no big toes; I have seen about 100 of these in a group high in the trees, this one fell about 15 m. from a tree and was badly injured, died 3 weeks later.' A snapshot-photograph of this little animal, also sent by Mr Carroll, bears an inscription claiming that the entire large group of chimpanzees seems to have been composed of 'very similar midgets, the largest not exceeding 40 lb'. This is mentioned merely because the particular specimen to be described is actually very small for its age, even though it is a mere infant and has as yet no permanent teeth. Incidentally, the writer cannot believe that anywhere near such large numbers of chimpanzees travel in groups and, of course, in no way endorses the rash claim of a 'new species'. This information, however, does give the interesting facts that this abnormal ape had been born in the wild, lived in captivity for only a very short time and had evidently been handicapped by the lack of thumbs and great toes, having

fallen out of a tree during the chase of the group. Of the injuries it supposedly received in the fall nothing could be detected and at least no part of the skeleton was in any way damaged by the accident. The latter fact could be determined with certainty from the numerous and excellent X-ray photographs of the specimen, for which the writer is very grateful to his colleague, Prof. H. R. Schinz, Director of the Röntgen Institute of the University of Zürich.

DESCRIPTION

A. Outer body

The well-preserved body of this infant chimpanzee bears a good coat of black hair, except for the usual few white hairs on the upper lip and chin and those other white hairs normally surrounding the anus of young chimpanzees. The fairly long hair of the scalp is limited to the parietal and upper occipital regions and the strikingly high forehead is nearly hairless, thereby accentuating the man-like appearance of the face. The skin is dark grey-brown on the head (including the entire face and ears), back and lateral surfaces of the limbs and it is light brown on the ventral side of the trunk and on the medial sides of the limbs, as well as on the palms and soles. The specimen, therefore, belongs to the dark-skinned variety or subspecies of *Pan troglodytes* and it is noteworthy that this mostly very dark pigment is already well developed in such a young specimen. This chimpanzee is a male and the testes are still (or again?) in the inguinal canals. According to its dentition it is infantile. All deciduous teeth have appeared and only the lower canines are not yet fully erupted. The fairly well formed crowns of the first permanent molars are still deep down in their alveoli. Two normal captive-born chimpanzees in the author's collection, with known ages of 1 year 7 months and 1 year 9 months respectively, show exactly the same conditions of their dentitions and correspond also very closely to the present specimen in regard to practically all details of skeletal maturation. It seems reasonable, therefore, to assume that the acrocephalic chimpanzee had also lived for at least one and a half years, but not for fully 2 years.

Table 1 lists some of the more important measurements and the significant body proportions of our abnormal chimpanzee and compares these data with the corresponding ones for twenty normal chimpanzees with full deciduous dentitions but as yet no permanent teeth. Sixteen of the latter are males and the other four females; all had been measured after their deaths by the writer himself strictly according to the methods described in detail in a special publication (Schultz, 1929 b). From these data it is first of all apparent that the acrocephalic specimen is much smaller than any of the normal infants in body weight and at best equal to the lowest values among the latter in regard to such main dimensions as sitting height, trunk height and chest girth. The abnormal infant may have lost weight during the 3 weeks of its captivity, though it does not look emaciated, but even in that case it is exceptionally small, since ten other normal infantile chimpanzees, for which the author could obtain the body weights at death, have an average weight of even 6.74 kg. and a minimum value of 5.22 kg. In spite of the fact that at all ages chimpanzees do vary to a surprising degree, especially in general body size (Schultz, 1954), it seems much more justified to regard our acrocephalic specimen as having been retarded

in its growth rather than as a representative of some new race of very small chimpanzees.

The body proportions selected for Table 1 show best in which respects the deformed infant deviates from the normal conditions and which main features have not become altered. The arithmetic mean of the length, breadth and height of the brain part of

Table 1. *Some absolute measurements and the most significant body proportions of the abnormal specimen and the averages and ranges of variations of the corresponding data for twenty normal infantile chimpanzees*

Absolute (mm.) and relative measurements	Abnormal specimen	Twenty normal specimens	
		Average	Range
Body weight in kg. (only fourteen normal specimens)	2.87	6.28	3.97-8.06
Sitting height	362	427.6	363-461
Trunk height	185	230.8	191-254
Chest circumference	325	413.1	325-484
Head length	97	119.4	112-130
Head breadth	85	101.0	93-110
Head height (from tragus)	68	70.5	64-78
Upper face height	44	58.3	51-63
Hand length	93	126.3	113-138
Foot length	95	134.7	113-148
Mean head diameter in % of trunk height	45.0	42.2	38.8-49.2
Upper face height in % of mean head diameter	52.7	60.2	53.4-65.2
Head height in % of head length	70.1	59.1	54.4-66.0
Interocular breadth in % of face breadth	24.7	18.3	15.4-21.4
Nose breadth in % of face breadth	19.5	38.5	32.5-42.7
Length of upper arm + forearm in % of trunk height	129.0	126.9	119.2-138.0
Length of thigh + leg in % of trunk height	117.2	116.6	108.2-124.5
Hand length in % of trunk height	50.2	54.9	49.2-61.6
Foot length in % of trunk height	51.3	58.4	55.0-63.8
Hand length in % of length of upper arm + forearm	38.9	43.2	40.3-47.1
Foot length in % of length of thigh + leg	43.7	50.1	47.5-52.4

the head, expressed in percentage of the trunk height (*suprasternale-sympyphion*), lies in the acrocephalus well within the normal range of variations of this index, indeed, in its relation to the trunk the brain of the acrocephalic specimen is of practically average normal size. From the second proportion in the table it is evident that the face part of the head, especially the height of the upper face (*nasion-stomion*), is in its relation to the brain part exceptionally small in the abnormal ape (see also Fig. 1). The third index expresses the most obvious characteristic of acrocephaly, the great proportionate height of the brain part, which equals 70 % of the head length in the abnormal infant, whereas on an average only 59 % in normal infants. The interocular breadth, relative to the face breadth (between the zygomatic arches), is significantly greater in the acrocephalic than in the normal chimpanzees, a condition which has been noted also in human acrocephalics (Park & Powers, 1920). Curiously enough the outer nose has only half the relative breadth in the deformed infant of that in the average normal one and in preparing the skull it had been found that the wing cartilages of the nose in the former are exceptionally small.

Two of the indices in Table 1 demonstrate that the lengths of the proximal and middle segments of the limbs in their relations to the trunk height are perfectly normal in the acrocephalus. The distal segments, however, are exceptionally short in relation to the trunk height and even more clearly in relation to the lengths of the

limbs without the hands and the feet respectively. It is evident, therefore, that the oligodactylysm of the deformed specimen is associated with some inhibition in the normal growth of those fingers and toes which had developed.

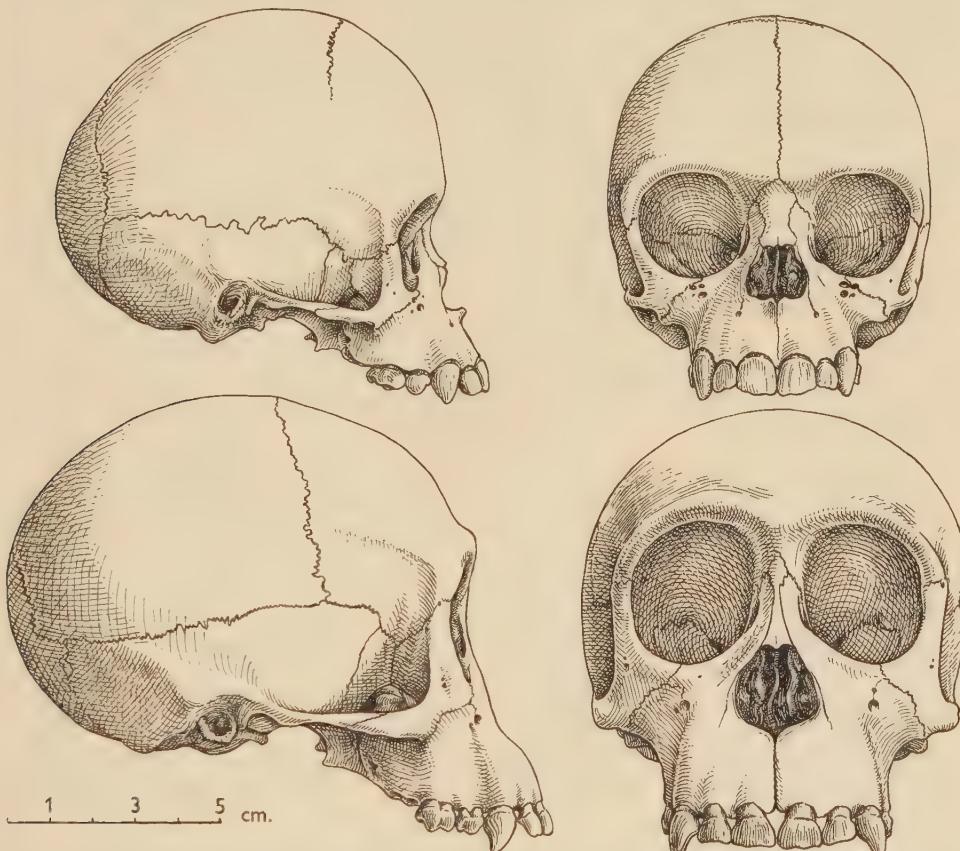


Fig. 1. Side and front views of the skull of the acrocephalic chimpanzee (above) and of a skull of a typical, normal, male West-African chimpanzee of corresponding age (below), both drawn by the writer to the same scale.

B. Skull

Since the acrocephalic skull contains so many abnormal and even unique features and in some ways approaches the conditions in human skulls so closely that it appears almost as a caricature of the latter, it deserves to be recorded in detail. The skull is illustrated in Fig. 1 and its main measurements are listed in Table 2. For comparison have been used sixteen normal skulls of chimpanzees of strictly corresponding stages of dental development and all belonging to the races of *Pan troglodytes* found in West Africa north of the Congo River. In addition, one infantile 'pygmy' chimpanzee (*P. t. paniscus*) from the left bank of the Congo River* could

* This rare skull had very kindly been lent to the author by the American Museum of Natural History in New York. Of the other sixteen normal skulls one belongs to the U.S. National Museum in Washington, two to the Anthropologische Institut of the University of Zürich and the remaining thirteen are from the author's collection.

be used and is listed separately in the table. Nearly all of the absolute cranial measurements are smaller in the arocephalus than in any of the normal chimpanzees including even the skull of *paniscus*. The exceptions are found in the height of the brain case, in the postorbital breadth (= 'minimum frontal breadth') and in the interorbital breadth. The first of the cranial indices in Table 2 confirms what had been shown by the corresponding index in Table 1, that in arocephaly the cranial vault is abnormally high for its length, whether measured from the ear opening, as on the outer body, or from the basion, as on the skull. The second cranial index

Table 2. Some absolute and relative cranial measurements of the abnormal specimen, of a normal infantile male 'pygmy' chimpanzee and of ten male and six female infantile West-African chimpanzees; for the latter, normal specimens only averages and ranges of variations are listed

(N.B. 'Mean diameter' is the arithmetic mean of the length, breadth and height of the neurocranium.)

Absolute and relative cranial measurements	Abnormal specimen	'Pygmy chimp.'	Sixteen normal western chimpanzees	
			Average	Range
Cranial capacity in cm. ³	231	252	333.9	274-425
Volume of one orbita	7	9	12.0	8.5- 15
Length (from glabella)	93	102	111.5	103-119
Breadth	79	84	93.5	87- 98
Height (from basion)	76	73	76.8	72- 86
Base length (basion-nasion)	62	64	69.9	66- 74
Minimum postorbital breadth	67	56	67.3	62- 73
Interorbital breadth	16	6	10.5	8.5- 12
Bizygomatic breadth	67	73	82.5	70- 91
Upper face height (nasion-prosthion)	40	48	54.0	47- 58
Palate breadth	35	38	43.4	39- 49
Height in % of length	81.7	71.5	68.9	62.3-76.7
Base length in % of mean diameter	74.8	74.1	74.4	70.5-78.2
Upper face height in % of mean diameter	48.3	55.7	57.5	53.0-62.0
Palate breadth in % of mean diameter	42.3	44.1	46.3	41.5-50.0
Orbital volume in % of cranial capacity	3.0	3.6	3.6	3.1-4.4
Postorbital breadth in % of skull breadth	84.8	66.7	72.0	66.7-78.5
Interorbital breadth in % of postorbital breadth	23.9	10.7	15.6	13.7-18.2

supports the conclusion that the skull base is not affected by the deformity, since at least in its relative length the base of the arocephalic skull equals that of the normal skulls. It may be mentioned here also that the spheno-occipital suture is still wide open in the abnormal skull as, of course, in all the normal skulls at this stage of development. Tables 1 and 2 both contain indices which express the height of the face without the lower jaw in relation to the mean diameter of the brain part of the head or skull and all these data agree in demonstrating the comparatively small size of the face in the arocephalus, which contributes to its man-like appearance. The orbits as well as the palate participate in this smallness of the face according to the indices in Table 2 which show the relative size of the orbital volume and of the palatine breadth respectively. The orbits of the small arocephalic specimen could have been expected to be relatively larger than in the normal specimens of greater general body size according to the relevant allometric rule (Schultz, 1940), but just the opposite condition exists. The orbits of the abnormal skull are particularly shallow in contrast to normal orbits of infantile chimpanzees, as indicated in Fig. 2, and undoubtedly for that reason the eyeballs of the arocephalic ape seemed

to protrude*. A corresponding exophthalmus has been noted in many of the human cases of acrocephalo-syndactylism in the literature. The palate as well as the lower jaw of the abnormal chimpanzee are smaller in absolute size than those of any of the normal infantile chimpanzees measured by the writer, and in relative size they

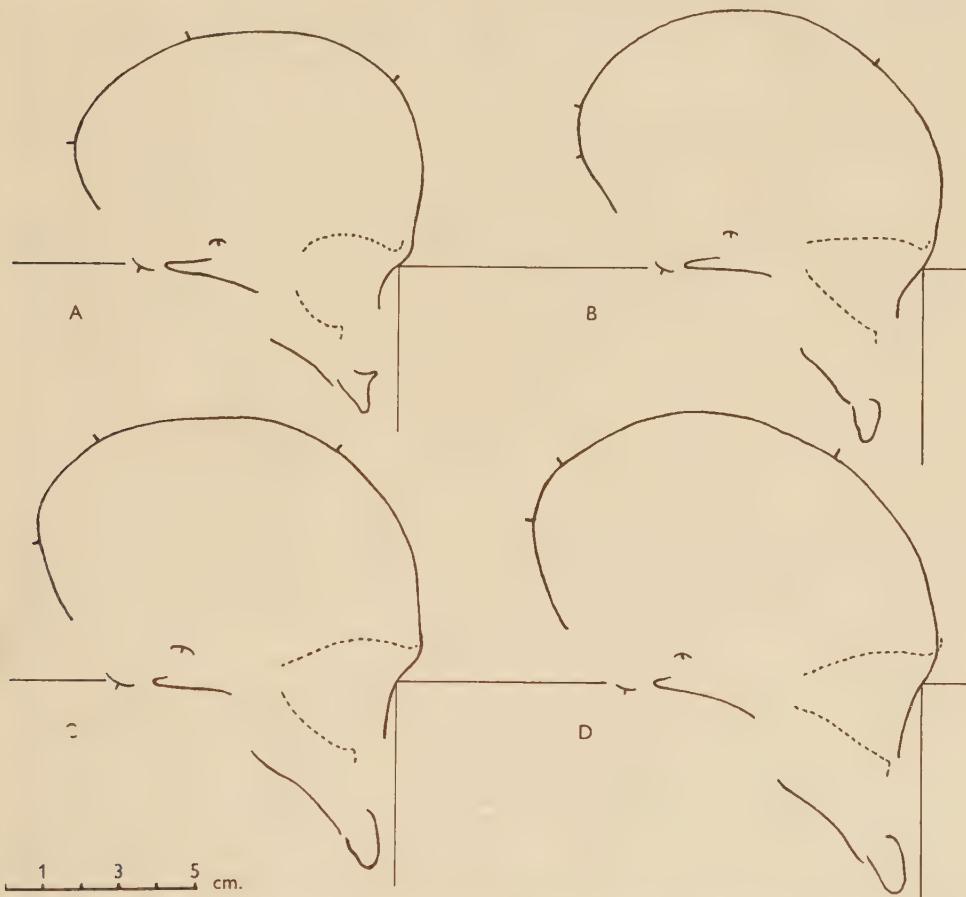


Fig. 2. Mid-sagittal cranial sections of male chimpanzees of corresponding ages, oriented in the nasion-basion horizon, showing location of nasion, bregma, lambda and inion and the projected location of porion, condylyon and of sagittal section of orbit. A = acrocephalus, B = normal 'pygmy' chimpanzee, C and D = normal West African chimpanzees.

barely equal the minimum values among the latter which include females. The teeth, however, are of fully average normal size in the acrocephalus and, hence, appear to be crowded in the small jaws and there is no trace of any diastemata. Again it can

* At least according to a small photograph of the living animal; in the preserved body the eyeballs had shrunk too much to retain the exact topographic conditions. The straight distance from the optic foramen to the nearest point on the lower orbital rim amounts to only 22 mm. in the acrocephalus, whereas it is at least 34 mm. in even the smallest of the normal infantile skulls. In its medial, lower and lateral parts the orbital entrance of the acrocephalus does not reach nearly as far forward and as close to the facial profile as in any of the normal infantile skulls. This is clearly shown by the lateral views in Fig. 1 and also evident from the sections in Fig. 2.

be mentioned that in nearly all the cases of 'acrocephalo-syndactylism' in man in which the palate was examined, it is described as extremely narrow and high (Park & Powers, 1920). In our abnormal ape the palate merely *seems* to be very high on account of its small size surrounded by teeth and alveolar processes of normal size.

The last two indices in Table 2 show clearly that the frontal region of the deformed specimen has grown in width to an exceptional degree and this not only between the temporal lines but also at its lower border. This, undoubtedly, is connected with the fact that the metopic or interfrontal suture has remained fully open, whereas normally this suture closes in chimpanzees long before the first dentition has fully erupted. The retention of the metopic suture in man is invariably accompanied by an increase in the width between the temporal lines as well as in the relative interorbital width, as has been demonstrated repeatedly (e.g. Schultz, 1929a, with review of previous relevant literature). The acrocephalic patient, described in detail by Park & Powers (1920) and measured by the writer, was also distinguished by an extraordinarily wide forehead and interocular region while the metopic suture was at least partly open. According to the last quoted publication the metopic suture remains open in the great majority of human acrocephalies in whom the abnormal condition had become evident early in life. It may not be mere chance, therefore, that our unique case of acrocephaly in an ape also has a fully open metopic suture, particularly since this suture had so far been found in only very few chimpanzees with more than *partially* erupted first dentition. At this point it must be emphasized that a suture which has remained open abnormally long tends to increase bone growth only in a direction perpendicular to its course, but not in the direction of its course, corresponding to the well-known opposite consequence of premature suture closure, found, for example, in scaphocephaly in which the early disappearance of the sagittal suture produces an abnormally narrow cranial vault.

Acrocephaly is bound to follow the early obliteration (if not lack of formation) of part or all of the right and left coronal sutures, which consistently tends to retard the *sagittal* growth of the frontal and parietal bones. As shown by Fig. 1, the acrocephalic skull lacks the lower parts of its coronal suture on both sides and consequently the vault is abnormally short. This retarded growth in length has been compensated by an increased growth in height and, most likely, has also favoured the retention of the metopic suture which permitted an increased frontal breadth of the brain case. The lack of a complete coronal suture in the acrocephalus has retarded, as expected, the *sagittal* growth of the frontal and parietal bones, but this deficiency is compensated by an increased growth of the occipital squama. These facts are apparent in the cranial sections in Fig. 2 which clearly show the extreme oral position of the bregma and lambda in the abnormal chimpanzee. The data in Table 3 also demonstrate this proportionately smaller length of the frontal and parietal and greater length of the occipital portions of the cranial vault in the acrocephalic than in the normal chimpanzees. From the last two columns in Table 3 it is evident that it is the part of the occipital above the inion that has come to contribute so much more to the formation of the cranial vault in the abnormal than in normal skulls. The lower half of Table 3 gives for comparison corresponding data for an adult human acrocephalus, contrasted with twelve normal adult human skulls from the same

locality. The results in this series agree with those found in the chimpanzees, but they are not as pronounced, even though the coronal suture of the human acrocephalic has almost completely disappeared (the lambdoid suture being still wide open). The writer inclines to the view that in this human acrocephalic the closure of the coronal suture, though clearly premature, had occurred at a comparatively later age than in our abnormal chimpanzee, if the lower parts of the coronal suture had ever existed in the latter.

Table 3. *Percentage participation of the frontal, parietal and occipital bones and of the parts of the occipital squama above and below the nuchal line in the formation of the total length of the mid-sagittal arc of the cranial vault*

(The adult human skulls are from an old village cemetery of Steinen (Kt. Schwyz), Switzerland.)

	Section of sagittal arc: ...	Nasion-bregma	Bregma-lambda	Lambda-opisthion	Lambda-inion	Inion-opisthion
Infantile chimpanzees	Acrocephalus	31.2	32.9	35.9	23.8	12.1
	Average of ten normal skulls	36.7	34.9	28.4	11.9	16.5
Adult Europeans	Acrocephalus	34.0	32.7	33.3	19.7	13.6
	Average of twelve normal skulls	34.9	34.2	30.9	17.6	13.3

As shown by Fig. 3, the frontal and parietal bones of chimpanzees develop normally, as in man, from ossification centres situated very near the middle of each of these bones. At foetal stages one can readily recognize these centres as dense and somewhat protruding zones from which fine lines radiate in all directions. In the acrocephalic specimen there are two zones of dense and perfectly smooth bone, lying close together far down in the right and left regions of the lacking coronal sutures, where they form slight protuberances. From these misplaced zones one can still see in good light faint radiating lines, indicating the direction of the earlier bony growth. It seems highly probable from this observation that no suture could ever have been formed between such adjacent ossification centres and that the acrocephaly really began with the early embryonic appearance of these ossification centres in their very abnormal location. This assumption is also supported by the old report of Zuckerkandl (1874) on a human newborn with extreme acrocephaly in whom the parietal ossification centre also lies practically in the course of the coronal suture, which however was formed only in its upper part, exactly as in our abnormal chimpanzee. It may be mentioned here also that the inhibiting influence of a partially missing or prematurely closed coronal suture on the growth of the skull is nowhere more clearly evident than in the cases of plagioccephaly, in which the coronal suture of only one side has been interfered with and which hence have become asymmetrical, one half being shorter and less vaulted than the opposite side. Quite a number of such plagioccephalic skulls of wild monkeys and apes have been recorded in the literature (Schultz, 1956). That ossification centres can occasionally become misplaced even so far that they interfere with the formation or closure of sutures, is no more surprising than the well-known and not at all rare duplication of normally single ossification centres, resulting in abnormally divided bones. Such malformations seem to occur particularly in the parietal bones, and incompletely or completely

divided parietals have been recorded for a very considerable number of wild monkeys and apes (Hrdlička, 1903; Schultz, 1956).

The acrocephalic skull of the chimpanzee contains some additional, very exceptional features, briefly to be described in the following: As shown by Fig. 1, there exists a well-formed nasal spine which projects forward for at least 2 mm. and had supported the cartilaginous nasal septum. The latter, however, was no more prominent than in normal chimpanzees and was flanked by unusually small wing

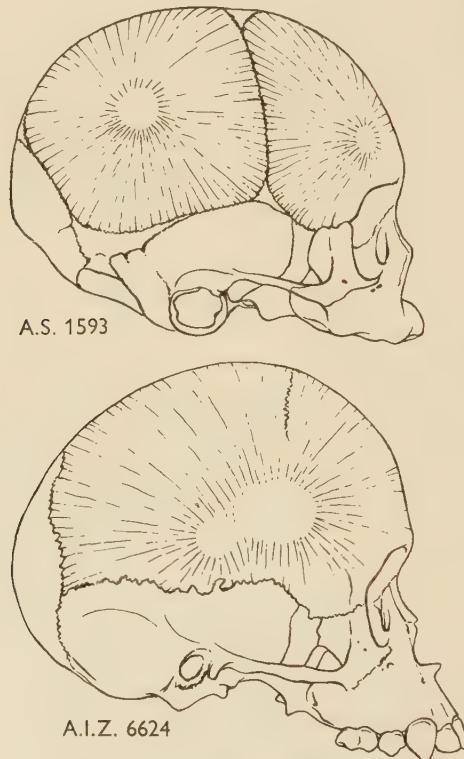


Fig. 3. Semidiagrammatic representation of the location of the ossification centres and of the direction of growth in the frontal and parietal bones of a foetal chimpanzee (above) and of the infantile acrocephalic chimpanzee (below).

cartilages, as has been mentioned before. As far as the writer knows, this is the first and only time that an unquestionable *spina nasalis* has ever been found in any non-human primate. The intermaxillary sutures extend from the lower end of the very broad and fused nasal bones to below the nasal aperture, where each suture ends abruptly in a fair-sized foramen (see Fig. 1) which had given passage to a blood-vessel. No such foramina could be found in any other chimpanzee. The last unique cranial feature to be mentioned is the apparently complete lack of the *lamina papyracea* of the ethmoid in the right and left medial orbital walls. In both these walls the frontal and maxillary bones meet behind the lacrimal, forming a straight suture extending all the way back to the palatine. Among 166 normal West African

chimpanzees the author (1952) had found a short post-lacrimal fronto-maxillary suture in 39 % of the cases and a lacrimo-ethmoid contact in the other 61 % and in all cases the ethmoidal plate was present.

C. Hands and feet

It has already been mentioned that the acrocephalic chimpanzee is also distinguished by the congenital lack of thumbs and great toes, i.e. by clear oligodactylysm, and that both its hands and feet are unusually short, though all the digits II to V are present and contain all normal parts. The exact conditions are illustrated in Fig. 4,

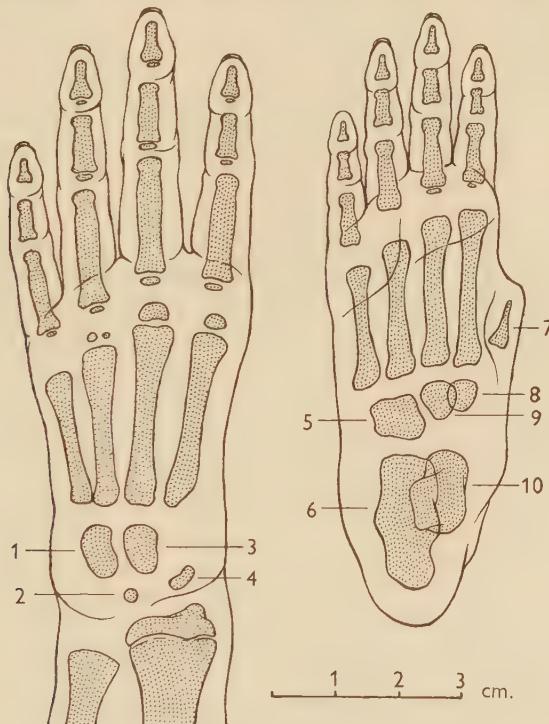


Fig. 4. Tracing of X-ray photographs (controlled by several different pictures) of the right hand and right foot of the infantile chimpanzee with oligodactylysm. 1 = hamatum, 2 = lunatum, 3 = capitatum, 4 = naviculare, 5 = cuboideum, 6 = calcaneus, 7 = vestigial metatarsus I, 8 = medial cuneiforme, 9 = lateral cuneiforme, 10 = talus.

and it is specially mentioned that these conditions are perfectly symmetrical in both hands and both feet and that there are no other abnormalities in either the upper or the lower extremities. The stage of ossification in the parts present seems to be perfectly normal for the age of dental development of this specimen, with the hands slightly more advanced than the feet as is to be expected.* The ossification of the hand and distal forearm corresponds very closely to that in a boy of 3 years

* The skeletal maturation of the abnormal specimen could be compared in detail for the entire body with the corresponding conditions in three normal infantile chimpanzees by means of X-ray photographs and it must suffice here to state that no noteworthy difference could be detected.

according to the standards in the atlas by Greulich & Pyle (1950). The congenital lack of the thumbs is complete and includes the metacarpals, whereas that of the great toes is incomplete since proximal rudiments of the first metatarsals are present and even forming slight protuberances on the outer surface of the feet.* This discrepancy in the degree of oligodactyly may quite likely be connected with the fact that the embryonic development of the fingers occurs slightly sooner than that of the toes and that, hence, any early disturbance in development could affect the hands more than the feet. According to the extensive literature on congenital malformations in the hands and feet of man the lack of the thumb occurs more often than that of the great toe, indeed, the isolated reduction of the latter is apparently almost unknown (Werthemann, 1952), and that of the thumb (specifically termed *aplasia of radialis I*) is not as frequent as most of the many other digital defects (Birch-Jensen, 1949). The same generalization can be made for non-human primates since many more cases of polydactyly, syndactyly and oligodactyly affecting the digits II to V have been recorded so far than incidences of abnormally reduced first digits (Schultz, 1956). As far as the author can ascertain, the only reductions in the great toes which had ever been found among monkeys or apes are the cases of congenital lack of the terminal phalanges in orang-utans in which this condition occurs in a majority of the many specimens examined, so that it cannot be regarded as really abnormal (Schultz, 1941). Congenital reductions in the thumb have been found in one guenon and two gibbons by the author and in one potto by Hill (1957); besides, of course, there are the 'normal' reductions of the thumb, characteristic of spider monkeys and of guerezas, in which, however, vestigial metacarpalia I are always retained and remnants of basal phalanges occasionally. From these remarks it is evident that the complete lack of both thumbs combined with the nearly complete loss of both great toes in our aerocephalic chimpanzee represents a very exceptional occurrence.

SUMMARY

It is the chief purpose of this report to place on record the description of a wild young chimpanzee which is distinguished by showing in combination a number of rare congenital malformations. The animal is exceptionally small for its age, as judged by its skeletal and dental development, and may therefore be regarded as retarded or stunted in its general growth. The localized anomalies have developed exclusively in the head and in both hands and feet and consist of the following conditions.

1. Abnormally high cranial vault (=aerocephaly) connected with complete lack of the lower halves of the right and left coronal sutures, the latter being most likely due to the abnormal position of the ossification centres for the frontal and parietal bones. These centres lie close together in the very region of the missing sutural part.
2. Abnormally wide forehead and interocular region due to the complete retention of the interfrontal suture.
3. Abnormally high occipital squama, particularly above the nuchal line, as compensation for the reduced sagittal growth of the frontal and parietal bones.

* Even though there is a knob-like elevation of the skin in place of the missing great toe and the metatarsal vestige does taper to a point, this is not a case of so-called spontaneous amputation, because no trace of scar formation can be detected and the malformation is far too symmetrical on both feet for such an explanation.

4. Proportionately small orbits, especially in depth.
5. Proportionately small face, including palate, but normal-sized teeth which in consequence are crowded.
6. Total lack of ethmoids on medial orbital walls.
7. Well-developed nasal spine, unique among non-human primates.
8. Total lack of both thumbs.
9. Lack of both first toes, except for vestigeal metatarsals.
10. Proportionately short hands and feet, though otherwise all the digits II to V are normally formed.

More or less similar deformities of the head have been found associated with various kinds and widely differing degrees of malformations in the hands and feet in human beings in so many instances that these combinations point to a common cause, namely the synchronous disturbance in the embryonic development of these particular bodily parts. This explanation appears particularly probable in view of the perfect symmetry of all these abnormalities.

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SOME ANATOMICAL FEATURES OF THE MANDIBLE

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MANDIBULAR CANAL AND MENTAL FORAMEN

In extreme resorption of the alveolar process of the edentulous mandible the mental foramen and part of the mandibular canal traversing the body of the bone may disappear and the inferior dental nerve come to lie upon the body of the bone in the soft tissues. Such a condition does not appear to have been described in anatomical literature. Gray's *Anatomy* (1954) states that when the mandible resorbs the mandibular canal and mental foramen are close to the alveolar border; Cunningham's *Text-book of Anatomy* (1951)--that 'in old age after the teeth are lost the walls of the sockets are absorbed and the mental foramen is near the upper border'. Morris's *Human Anatomy* (1942), Quain's *Elements of Anatomy* (1890) and the *Text-book of Anatomy* edited by Hamilton (1956), merely mention senile absorption of the alveolar margin. In Cowdry's *Problems of the Ageing* (1952) it is stated that the mandibular canal in the elderly is near the alveolar surface and that the mental foramen appears near or even on the alveolar surface. Last (1954) also indicates that in the edentulous jaw the mental foramen lies nearer the upper border of the mandible. Humphrey (1858), who gives a more complete description than many modern text-books, says that in edentulous old age the inferior dental canal runs along the alveolar border and that the mental foramen comes to lie on its upper edge. Cogswell (1942) does not appear to envisage the nerve lying outside the bone, though the conditions he dealt with mainly concerned the dentulous bone. Edwards (1954) complains of lack of description of the edentulous mandible and states that the mental foramen comes to lie near the ridge. He illustrates what he regards as an extremely resorbed bone which shows, however, the mental foramen still present. Augier (1928) describes the bone as becoming resorbed almost to the mental foramen. Le Double (1906) says that when the teeth fall out the alveolar process is lost and the mandible is reduced to its body. He states later his belief that no one has reported absence of these foramina: 'Je ne crois pas qu'on ait encore fait mention de l'absence des ouvertures en question. En ce qui me concerne, je les ai toujours rencontrées toutes les deux, sur chacune des 822 mâchoires inférieures.' Praed (1938), however, writes: 'The ridge may be flattened throughout, even involving the mental foramen on one or both sides.'

Surgeons have encountered the nerve lying exposed in the soft tissues (Wallace, 1956), and Gerry (1956), reporting a case of a patient 40 years of age who had been edentulous for 20 years, writes: 'the inferior alveolar vessels were not covered by bone in the bicuspid region where the mandible was only 3-4 mm. thick'. It is possible that in such cases the condition has been regarded as pathological.

Two mandibles are illustrated here which show the disappearance of the mental foramen and part of the mandibular canal. The first specimen (Pl. 1, figs. 1, 2) was

recovered from the dissection room of the Medical School at Sydney University. The body of the bone varies in thickness from 6 to 13 mm. In the second and third molar region and where the upper surface of the body slopes up into the ramus the inferior alveolar nerve was covered by a thin lamina of bone but more anteriorly the mandibular canal was transformed into a groove. This groove became shallow and indistinct in the premolar region. Branches of the nerve passed into the bone, one large branch may be seen in the lateral view of the bone (Pl. 1, fig. 1) entering the bone in the canine region, but the main trunk of the nerve lay along the surface of the bone. This bone shows fairly uniform resorption throughout the body.

The specimen illustrated in Pl. 1, figs. 3 and 4, was a macerated bone which had suffered considerable resorption in the anterior region but possessed a high and sharp ridge posteriorly. This bone had been sectioned transversely for another purpose some years ago (Gabriel, 1946). The sections have been replaced in their correct positions and photographed. Pl. 1, fig. 3 shows that the mental foramen has disappeared. The crano-caudal view (Pl. 1, fig. 4) shows the canal opening above the upper surface of the much resorbed anterior part of the bone.

In the adult dentulous mandible the mandibular canal, as it passes forward in the body of the bone, lies several millimetres below the level of the mental foramen which it eventually reaches by bending acutely below it and then passing upward (Gabriel, 1946). Hence a considerable amount of bone must be resorbed from the mandible below the level of the mental foramen before the main part of the mandibular canal is exposed.

The following question arises. Does the level or height of the mental foramen relative to the lower border of the mandible alter in post maturity? To test this, measurements of the perpendicular distance of the mental foramen to the lower border were made on 21 dentate, 21 partly edentulous, and 21 edentulous mandibles, and the mean measurement for each group was found to be 13.8, 13.0 and 13.0 mm. respectively. By the usual variance ratio test, the difference between these means is not significant, the variance ratio being 1.80 for $n_1=2$ and $n_2=60$ in the usual notation.

Mandibular foramen

The position and variation of the mental foramen has, over the years, received much attention and there is a considerable volume of literature describing this feature but much less work has been done to describe the mandibular foramen. The position of the mandibular foramen is difficult to define. This difficulty is due mainly, it would seem, to the variability of the angle of the jaw. Many authorities (e.g. Dixon, 1912) have referred loosely to the foramen as lying approximately in the centre of the medial surface of the ramus of the mandible, and, indeed, this statement in many cases proves almost as accurate as the more involved definitions given. Fawcett (1895) writes: 'the canal commences in the ramus midway between its anterior and posterior borders, and at the point of junction of the lower third with the upper two-thirds of a line drawn from the tip of the coronoid process to the angle of the jaw', and further that 'this line passes through the base of the lingula and the beginning of the inferior dental canal or groove leading into it, midway between the anterior and posterior borders of the ramus'. The relation of this line to the lingula has been examined on some thirty bones and up to 10 mm. variation

has been found. The lingula was more often in front of than behind the line. The tip of the coronoid process could be determined with reasonable accuracy and 'the angle' was assumed to be the point of intersection of a line tangential to the lower border of the body (basal plane) with a line tangential to the posterior border of the ramus (rameal plane). No attempt was made to confirm Fawcett's other statement as it was obviously intended to be merely an approximation. Miller (1953), dealing with dentate bones, related the mandibular foramen as from 31 to 58 % of the distance between the mandibular notch and the gonial notch and from 45 to 71 % of the width of the ramus posterior to the anterior border of the ramus. These figures indicate the wide variation to be found.

Lotric (1951) states that the lowest point of the mandibular foramen is on an average 20–25 mm. from the *incisura mandibularis* and about 20 mm. behind the anterior margin of the ramus and that these ratios are practically constant. He states, further, that the foramen (lowest point?) is in 82 % of cases below the occlusal plane of the lower molar teeth, in 13 % of cases on the same level and only in rare cases above it. Unfortunately Lotric's original paper was not available and the above statement was taken from an abridged account given in *Excerpta Medica* (1951). Augier (1928) describes the mandibular foramen as situated about 2 cm. from the anterior border of the ramus, about midway between the anterior and posterior borders and a little above the level of the teeth. The level mentioned seems somewhat in conflict with Lotric's statement but might be explained by Augier having adopted a different point of reference on the foramen or by the difference between what was meant by 'the level of the teeth' in one case and 'the occlusal plane of the lower molar teeth' in the other.

The writer cannot improve on these definitions of the position of the mandibular foramen, but after making a series of measurements (see Appendix and 'Definition of Measurements') he finds some correlation of its position with the angle of the mandible and the breadth of the ramus as follows:

(1) A negative correlation of -0.823^* was found between measurements of the angle of the mandible and the perpendicular distance between the mandibular foramen and the lower border of the bone which we will call the 'height' of this foramen.

(2) A negative correlation of -0.649^* was found between the measurement of the angle of the mandible and the distance between the mandibular foramen and the posterior border of the ramus.

(3) A negative correlation of -0.426^* was found between measurements of the angle of the mandible and the minimum breadth of the ramus.

These correlations indicate that the smaller the angle of a mandible, or in other words the more upright its ramus, then the higher will be the mandibular foramen, the greater will be the distance of this foramen forward from the posterior border, and the broader will be the ramus.

Due to the fact that these measurements were of a mixed collection of bones including juvenile and senile, dentulous and edentulous, it might reasonably be inferred that the negative correlation found between the breadth of the ramus and angle of the mandible was merely an expression of the fact that both juvenile

* Each of these coefficients of correlation is significant beyond the level of 0.1%.

mandibles and senile edentulous mandibles may be expected to have, coexisting, slender rami and large angles. To test this matter calculations were made of a series of dentulous adult mandibles. Harrower (1928) in his biometric study of 110 Asiatic mandibles provides excellent material for this purpose and his paper records all the individual measurements he made. Analysis of his relevant measurements of the angle and the rami of those mandibles gives a coefficient of negative correlation of -0.512 (significant beyond the level of 0.1%) which confirms the validity of this correlation and clarifies the point.

Although not arriving at the conclusion that the height of the mandibular foramen is related to the angle of the mandible, Allen (1915), in his text-book of local anaesthesia, appreciated that in youth the mandibular foramen lies at a lower level relative to the teeth than it does in the adult. This is indicated by his illustrations showing the direction in which the needle of a local anaesthetic syringe should be advanced in the respective cases of a child, a youth and an adult; and also by his definition of the position of the mandibular foramen which is as follows: 'The mandibular foramen is situated about the middle of the internal surface of the ascending ramus, and in the adult is above the alveolar ridge and in a horizontal plane about 1.5 cm. from the anterior ridge.'

DEFINITIONS OF MEASUREMENTS

Mental foramen. None of the bones examined possessed more than one mental foramen on each side. The lower border of the mental foramen is the part most clearly defined, and measurements were taken from the lowest point on this border.

Mental foramen to lower border is the distance from the above point of reference on the mental foramen to the lower border of the bone perpendicularly below.

Mandibular foramen. The point of reference is the lowest point of the lower border where it merges into the lingula.

Mandibular foramen to lower border is the perpendicular distance from the reference point on the foramen to the lower border of the bone.

Mandibular foramen to posterior border of ramus is the distance from the reference point on the foramen to the nearest point on the posterior border.

Minimum breadth of ramus is the minimum distance between the anterior and posterior borders of the ramus. This is usually at or about the level of the mandibular foramen.

Standard basal plane (after Morant) is a horizontal plane with which the lower border of a mandible makes the most contact when vertical pressure is applied to the regions of the second molar teeth.

Rameal plane. A plane which makes tangential contact with the two most prominent convexities of the posterior border of the ramus.

Angle of the mandible or gonial angle is measured at the intersection of the standard basal plane with the rameal plane.

Perpendicular measurements are perpendicular to the standard basal plane.

SUMMARY

Resorption of the alveolar process of the edentulous mandible may progress to elimination of the mental foramen and part of the mandibular canal.

Correlations of measurements indicate that the more upright the ramus of a mandible then the higher will be the mandibular foramen, the greater will be the distance of this foramen forward from the posterior border, and the broader will be the ramus of the mandible.

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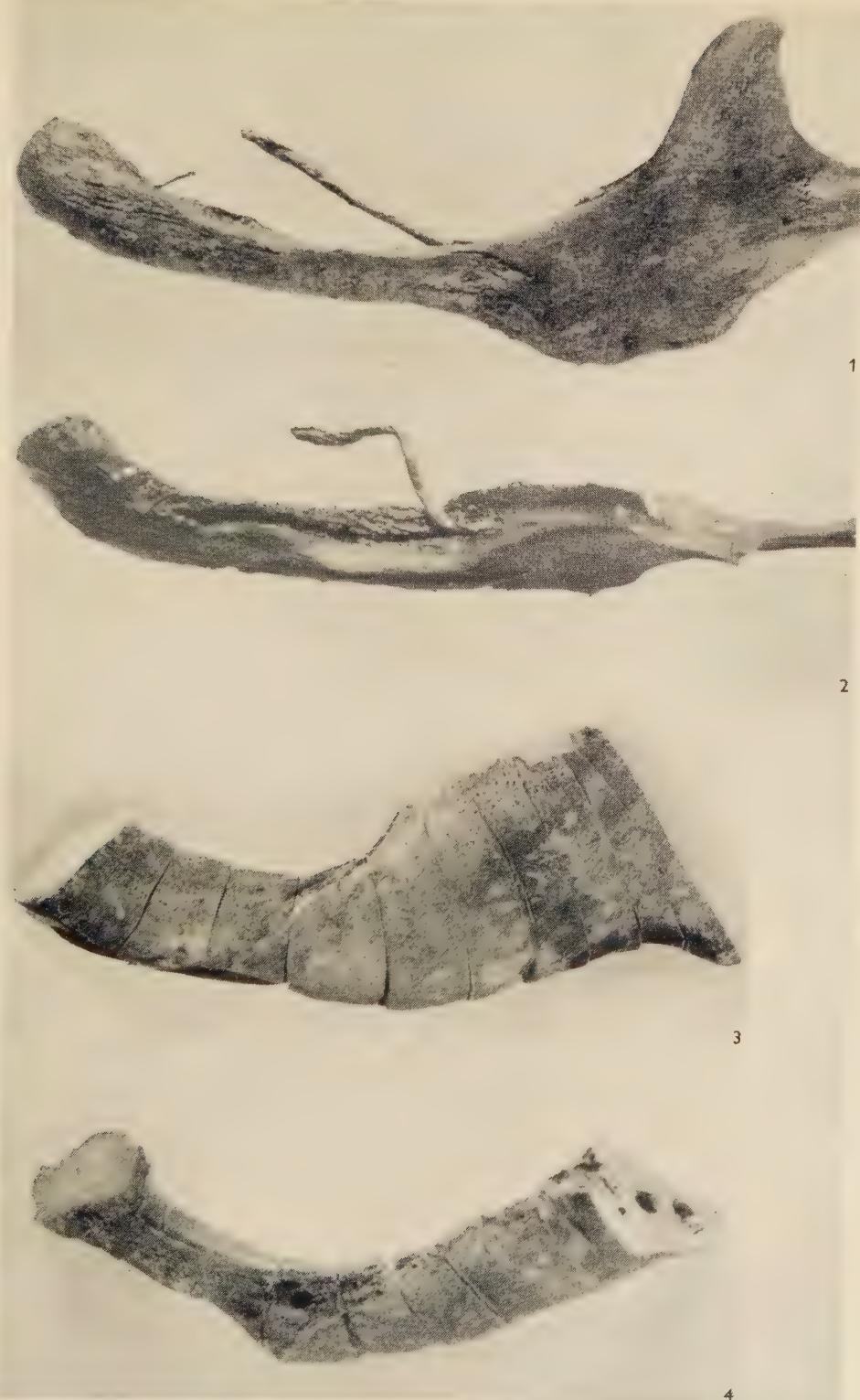
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EXPLANATION OF PLATE

Lateral and superior views of the left side (Figs. 1 and 2) and part of the left side (Figs. 3 and 4) of two human mandibles, showing absorption of the alveolar process to below the level of the mental foramen.



APPENDIX

Mandible	Side	Mandibular foramen to lower border (mm.)	Mandibular foramen to posterior border (mm.)	Minimum breadth of ramus (mm.)	Angle of mandible (°)
A.D. 13	L.	13	9	22	133
	R.	14	10	23	135
921	L.	16	12	28	129
	R.	17	12	27	125
801	L.	21	11	28	129
	R.	19	11	28	129
226 B	L.	20	15	34	124
	R.	20	16	33	122
471	L.	17	12	28	138
	R.	17	11	27	138
1	L.	20	9	25	127
	R.	19	11	26	127
3	L.	27	14	32	114
	R.	28	16	32	115
8	L.	16	12	22	135
	R.	15	10	20	140
15	L.	20	14	28	134
	R.	23	15	28	135
4	L.	19	13	24	135
	R.	17	13	22	146
2	L.	27	18	34	106
	R.	24	15	32	108
5	L.	17	11	28	136
	R.	15	11	27	140
7	L.	17	13	27	130
	R.	18	13	27	131
11	L.	22	18	27	118
	R.	21	18	28	120
Jap.	L.	20	20	33	115
	R.	20	20	34	118
800	L.	11	12	25	135
	R.	9	10	24	141
914	L.	25	15	35	115
	R.	25	16	36	110
W.M. 92	L.	25	13	34	113
	R.	27	14	35	113
93	L.	17	14	29	137
	R.	16	13	31	136
94	L.	22	21	35	111
	R.	24	19	35	112
96	L.	20	15	34	123
	R.	21	15	34	121
97	L.	20	13	32	120
	R.	19	12	32	121
107	L.	28	16	35	116
	R.	27	16	34	117
108	L.	28	13	31	111
	R.	27	14	30	114
228	L.	18	15	34	125
	R.	20	14	34	124
229	L.	20	11	30	124
	R.	20	12	29	126

Mandible	Side	Mandibular foramen to lower border (mm.)	Mandibular foramen to posterior border (mm.)	Minimum breadth of ramus (mm.)	Angle of mandible (°)
W.M. 195	L.	24	14	36	120
	R.	27	15	36	116
196	L.	26	16	39	113
	R.	29	17	40	113
187	L.	24	15	31	114
	R.	24	16	31	112
189	L.	28	15	32	110
	R.	28	15	31	109
31	L.	17	16	37	122
	R.	18	16	38	123
32	L.	25	17	30	112
	R.	24	17	30	112
18	L.	15	10	21	130
	R.	28	17	38	121
118	L.	34	17	36	108
	R.	31	15	33	107
150	L.	27	14	35	116
	R.	28	14	36	116
151	L.	21	13	29	125
	R.	22	15	30	122
153	L.	28	16	34	109
	R.	28	16	33	109
154	L.	27	17	35	120
	R.	26	17	37	120
81	L.	20	14	30	125
	R.	20	14	30	125
85	L.	20	15	33	127
	R.	21	14	33	125
86	L.	15	10	27	138
	R.	14	12	25	140
87	L.	18	13	25	134
	R.	19	13	24	133
88	L.	19	14	30	128
	R.	21	14	31	125
91	L.	18	14	30	125
	R.	17	14	29	126
110	L.	21	14	33	117
	R.	22	14	32	119
142	L.	26	14	31	118
	R.	30	15	34	118
146	L.	18	14	34	127
	R.	18	14	33	122
147	L.	23	20	36	110
	R.	26	19	37	118
152	L.	20	10	31	122
	R.	23	10	31	122
252	L.	19	14	34	126
	R.	24	15	35	126
Summary Means		21.49	14.20	30.98	122.72
Standard deviation		4.75	2.55	4.31	2.96
Standard error of means		0.48	0.25	0.43	0.30

THE TUBERCLE OF THE TIBIA

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Anatomical and orthopaedic text-books (Last, 1954; Frazer, 1946; Watson-Jones, 1955) usually describe the insertion of the ligamentum patellae as being into the upper smooth part of the tibial tubercle. This smooth prominence is limited below by a line directed downwards and outwards representing, as is well known, the position of the epiphyseal line, and thus, according to these descriptions, the ligamentum patellae is attached only to the upper tibial epiphysis. Gray (1954) gives a similar description, but notes that the line representing the lower limit of the smooth epiphyseal area is sometimes represented by a rough crest which divides the tubercle into upper smooth and lower rough portions, and receives the superficial fibres of the ligament. The attachment is, however, here figured extending slightly on to the diaphysis, but this is presumably inadvertent rather than by design. This rough crest or ridge giving attachment to the ligamentum patellae is also noted by Humphry (1858). Other text-books (Schafer, Symington & Bryce, 1915; Boyd, Le Gros Clark, Hamilton, Yoffey, Zuckerman & Appleton, 1956) state that the ligamentum patellae is attached to the distal rough part of the tibial tubercle.

The findings at dissection and the varying form of the tibial tubercle seen in dry bones cannot be clearly interpreted in the light of such descriptions. It is commonly seen in the dissecting room that a thick superficial layer of the ligamentum patellae continues below the lower limit of the smooth epiphyseal elevation downwards for 1–2 cm. on to the shaft before terminating at a transversely disposed ridge or step raised on the anterior aspect of the shaft. This ridge may be directed rather obliquely downwards and outwards, but not invariably so. In such subjects, if the ligamentum patellae is pulled distally it readily tears from its attachment to the smooth area (leaving a depression on the deep surface of the tendon representing this attachment) but remains firmly attached to the bone by its lower diaphysial attachment. Fig. 1 A, B, represents the appearance of the bone and the region of tendon attachment in such cases. A longitudinal section of the tibia in this type (Fig. 2 D) shows that the fibres of the deeper part of the tendon pass into the bone of the smooth epiphyseal area of the tibial tubercle, while the more superficial part of the tendon continues down the shaft, fibres progressively leaving its deep surface to enter the bone and finally most of the remaining fibres continue into the bone of the step-like ridge (Fig. 1 A, c). A few of the most superficial fibres merge with the periosteum below this. As commonly occurs with other tendons, the dense collagenous tissue of the ligamentum patellae is frequently modified into fibro-cartilage before passing into the matrix of the bone. Testut (1904) figures a very similar attachment of the ligamentum patellae to that shown in Fig. 1 A but, in the text, describes the attachment as being to the lower part of the tibial tubercle.

In other dissecting-room subjects the insertion of the ligamentum patellae is only to the smooth epiphysial area as described in most text-books (Fig. 1 B). The smooth prominence may, however, be limited below by a rough crest or step-like ridge receiving the superficial fibres of the ligamentum patellae, and this ridge will be shown to be of diaphysial origin. Examination of twenty-three dissecting-room specimens and twenty-four dry bones indicates that these alternative types of attachment of the ligamentum patellae (Fig. 1) are of approximately equal distribution.

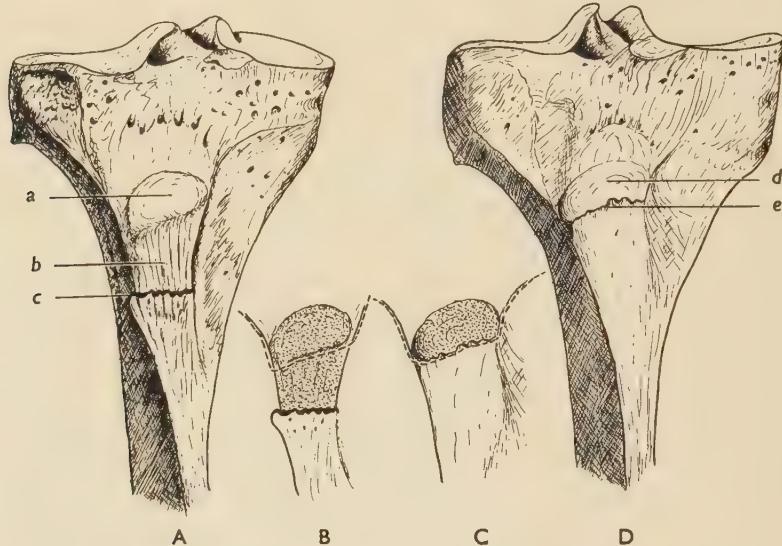


Fig. 1. A is a tibia of the type in which the ligamentum patellae is attached to epiphysis and diaphysis; *a* is the smooth epiphysial part of the tibial tubercle, *b* the lower ridged and roughened diaphysial part terminating below in a crest or ridge, *c*. In B the area of attachment of the ligamentum patellae on such a bone is stippled and the position of the epiphysial line is indicated by a broken line. D represents a tibia of the type in which the attachment of the ligamentum patellae is almost entirely to epiphysis; *d* is the smooth epiphysial prominence of the tubercle limited below by the crest *e*. In C the area of attachment of the ligamentum patellae on such a bone is stippled and the epiphysial line is shown by a broken line.

The explanation of these variations can be found in the structure of this region in the new-born child. Here the primary attachment of the ligamentum patellae is clearly to the diaphysis. It passes over the small raised cartilaginous promontory which will become the smooth area of the tibial tubercle, gaining some attachment by its deep surface to it, on its way to be attached to the anterior subcutaneous border of the tibia. An instructive dissection can be made by pulling the severed ligamentum patellae distally—it readily separates from the cartilaginous smooth area of the tubercle, leaving a depressed area representing this attachment on the deep surface of the ligament, but remains firmly attached to the anterior border of the shaft which is clearly its primary attachment. Fig. 2 C is a drawing of a longitudinal section down the leg of a still-born foetus. The epiphysial portion of the tibial tubercle is seen as a cartilaginous tongue extending forwards and down-

wards into the deep surface of the ligamentum patellae. The process of chondrification of the upper extremity of the tibia is clearly extending into the deep aspect of the ligamentum patellae, whose primary attachment has obviously been to the diaphysis. The deep fibres of the ligament pass into and blend with the matrix of this cartilaginous tongue from above; similarly, fibres leave the deep aspect of the tongue below to join it to the diaphysis. The superficial fibres of the ligamentum patellae pass without interruption over this cartilaginous tongue to be attached to the diaphysis. There is a gradual transition, both superficially and deeply, between the dense collagenous tissue of the deeper part of the ligamentum patellae and the cartilage of the tongue. Also a significant fact emerges—while the great mass of the cartilaginous upper extremity of the tibia is joined to the diaphysis by typical growth cartilage with its columns of hypertrophic cartilage cells, the lower and deep aspect of the tongue is connected to the bone of the diaphysis by dense collagenous ligamentous tissue. The portion of the ligamentum patellae passing superficial to the tongue and also the fibres passing from the deep aspect of the tongue merge below with the upward and forwardly directed trabeculae of the shaft and there is a gradual transition between ligament and bone. Intramembranous ossification is clearly extending upwards here, into this ligamentous tissue. The derivation of the adult arrangements from this structure is clear. The cartilaginous upper extremity of the tibia of the full-term foetus becomes the upper epiphysis with its tongue-like anterior projection now ossified. This tongue may be ossified by extension from the main tibial epiphysis, or in 50 % of cases have an independent centre of its own fusing shortly with the main epiphysis (Francis, 1940). Union of the greater part of the compound epiphysis so formed is across a cartilaginous epiphyseal plate but union of the deep aspect of the tongue-like extension must be by intramembranous ossification in the fibrous tissue connecting it to the diaphysis. This now gives rise to the appearance seen in Fig. 1 A, the step-like ridge being the point where ossification into the lower ligamentous fibres has ceased; when this ossification encroaches further up in this ligamentous tissue to the lower margin of the epiphysis an arrangement such as is shown in Fig. 1 D is produced. The downwardly extending cartilaginous tongue of the full-term foetus has, except for its continuity with the main mass of the cartilaginous upper extremity of the tibia, all the features of a sesamoid structure developing in the deep aspect of the ligamentum patellae whose primary attachment is to the diaphysis.

Considerable evidence for a sesamoid origin of the upper smooth part of the tibial tubercle is obtained from comparative study. A sagittal section down the knee joint of a pouch embryo of the black-tailed wallaby, *Macropus ualabatus* (Fig. 2 B), shows an appearance similar to the human embryo. Here, however, that part of the upper extremity of the tibia set in the course of the deep part of the ligamentum patellae has a large secondary centre of ossification, is larger, and a groove partially limits it from the main part of the upper extremity with its pressure epiphysis; otherwise the arrangements are as in the human foetus. The appearance could be readily explained if an earlier stage such as Fig. 2 A is postulated in which a free sesamoid bone is developed in the ligamentum patellae, where it passes anterior to the upper epiphysis of the tibia, which later fuses to the pressure epiphysis of the tibia. A similar arrangement to the wallaby has been described by Lacroix (1949)

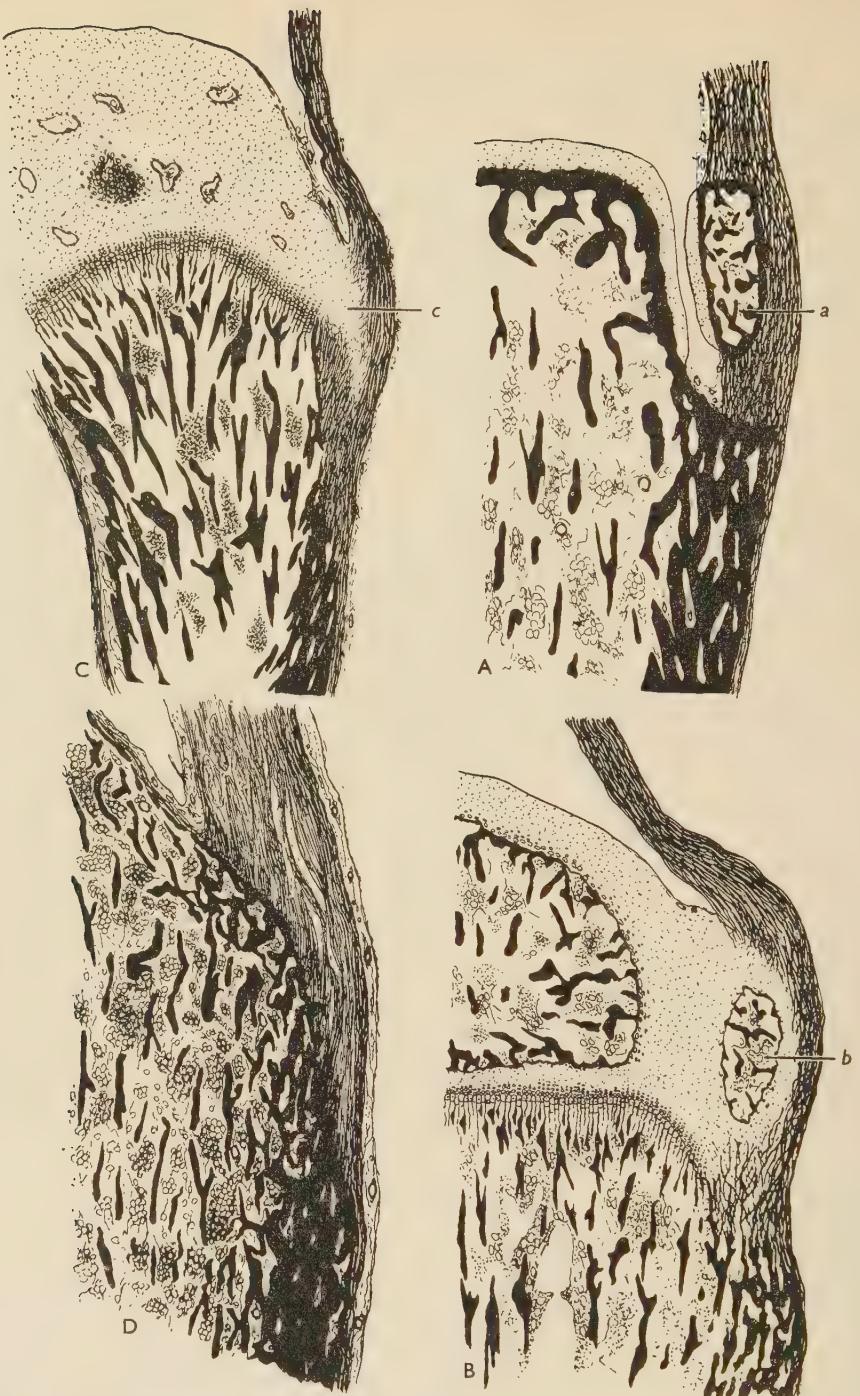


Fig. 2. A is a hypothetical sagittal section of an animal in which the precursor of the upper part of the tibial tubercle is present as a sesamoid bone (*a*) in the ligamentum patellae. B is a drawing of a sagittal section of the upper end of the tibia of a pouch embryo of the black-tailed wallaby. The secondary centre of ossification, *b*, lies in that part of the cartilaginous upper extremity of the tibia developed in the course of the ligamentum patellae, and is anterior to the upper pressure epiphysis of the tibia. C is a drawing of a sagittal section of the upper end of the tibia of a full-term human foetus showing the cartilaginous tongue, *c*, extending into the deep part of the ligamentum patellae. D is a sagittal section of the tibial tubercle of an adult, with a tibia of the type shown as A in Fig. 1.

and Bidder (1906) in the young rabbit and by McLean & Bloom (1940) in the rat, though here there is merely independent calcification of the cartilage and not a true secondary centre of ossification for the upper part of the tibial tubercle.

The probable sesamoid origin of the upper smooth part of the human tibial tubercle is even more clearly demonstrated in the lizard *Uromastix spinifer*. In this species the upper pressure epiphysis of the tibia is restricted to the anterior part of the upper extremity of the tibia (and is not to be confused with the epiphysis for the tibial tubercle in, for example, the black-tailed wallaby). However, projecting from the anterior aspect of the epiphysis into the deep part of the ligamentum patellae is a cartilaginous tongue somewhat similar to that of the human full-term foetus, but here its attachment to the upper tibial epiphysis is much more restricted and its original independent origin appears extremely likely. In the frog, where true epiphyses do not occur, although superficial calcification is present in the cartilaginous extremities (Parsons, 1905), the ligamentum patellae, as would be expected, passes to the diaphysis. While no example of a separate sesamoid structure in the ligamentum patellae has been seen there is little doubt, from the examples described, that there was originally such a structure which later fused to the pressure epiphysis of the upper end of the tibia. Although the upper smooth part of the human tibial tubercle often has a separate centre of ossification it does not appear to chondrify separately. Thus, even in a 7 cm. human foetus, the cartilaginous tongue noted at full-term is apparently developing by extension of the process of chondrification into the deep part of the ligamentum patellae.

CONCLUSIONS

Parsons (1908) has maintained that all traction epiphyses are probably derived from sesamoid structures, a view which has, however, been denied by Haines (1940). Parsons states: 'when a sesamoid structure in a tendon is caused by the pressure of a bone into which the tendon is on its way to be attached, without the intervention of a fleshy belly, it fuses with that bone and a traction epiphysis results. When, on the other hand, the tendon is on its way to be attached to another bone, the sesamoid structure remains permanently in that condition'. A consideration of the adult, foetal and comparative attachments of the ligamentum patellae makes it seem very likely that the upper smooth part of the human tibial tubercle was originally a sesamoid structure.

Watson-Jones (1955) has stated that Osgood-Schlatter's disease is due to the pull of the ligamentum patellae, whose sole insertion is to the epiphysis, straining the epiphyseal line of the tubercle, the weak point in the extensor mechanism of the knee. Reference to Fig. 2 C shows that the condition must actually consist of partial disruption of the *ligamentous* attachment between the tibial tubercle and the diaphysis, i.e. that part of the ligamentum patellae below and deep to the forward and downward projecting tongue of the tibial epiphysis. Watson-Jones also mentions a case in which, after repeated strains and continuous pain, the tubercle was found attached to the tibia only by fibrous tissue at the age of 40 years. This fibrous tissue obviously does not represent a new connexion between the tubercle and the remainder of the tibia following disruption of the epiphyseal line and non-union, but consists

of the fibrous union present from the earliest stages, in which ossification has not occurred due to the frequent tensional strains.

As the bone grows, ossification proceeds upwards into the attachment of the ligamentum patellae to the diaphysis. This gives rise to the strong anterior subcutaneous border or crest of the bone which receives the pull of the quadriceps femoris muscle. It also forms the cnemial crest in those animals possessing one (e.g. dog, wallaby).

SUMMARY

1. The ligamentum patellae of the adult may be attached almost entirely to the upper tibial epiphysis or partly to epiphysis and diaphysis.
2. The upper, smooth, epiphyseal part of the tibial tubercle develops by chondrification in the deep part of the ligamentum patellae on its way to the diaphysis; there is evidence to suggest that it had a sesamoid origin phylogenetically.
3. In Osgood-Schlatter's disease it is almost certainly the fibrous union between the epiphyseal part of the tibial tubercle and the diaphysis which is partially disrupted and not a cartilaginous epiphyseal plate.

I should like to thank Prof. D. V. Davies for his helpful advice, and Mr A. V. Freeborn for technical assistance.

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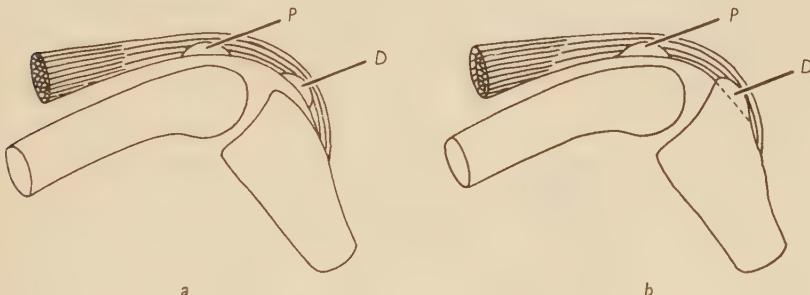
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THE EVOLUTION OF SOME TRACTION EPIPHYESSES IN BIRDS AND MAMMALS

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Parsons (1904) introduced the term 'traction epiphysis' to denote a projection, usually from one end of the shaft of a bone, into which a tendon is inserted and which has a separate centre of ossification. He suggested (1904, 1908) that many of these epiphyses represented pre-existing intratendinous sesamoids, which had become secondarily fused to the main bone. His explanation for this evolutionary change may be summarized as follows. A tendon passing over a flexed joint tends to develop proximal and distal sesamoids (*P* and *D*, Text-fig. 1*a*) where the tendon rubs over the corresponding bones; in either situation more than one sesamoid may be developed. A distal sesamoid (or sesamoids) would move only slightly relative to



Text-fig. 1. *a*, proximal (*P*) and distal (*D*) sesamoids related to a hypothetical joint;
b, conversion of distal sesamoid into a traction epiphysis.

the distal bone of the joint, however, and would therefore tend to fuse with it, the point of action of the tendon being transferred from its original insertion to the fused sesamoid itself, now a traction epiphysis (Text-fig. 1*b*). Pearson & Davin (1921*a, b*), on the other hand, reviewed the literature concerning the sesamoids around the knee and from their own extensive studies concluded that sesamoid bones arose by the separation of bony processes, a view opposite to that of Parsons. Haines (1940, 1942*b*) disagreed with both theories, stating that traction epiphyses and sesamoids had evolved independently.

A number of mammalian and avian joints have been examined in an attempt to resolve this controversy.

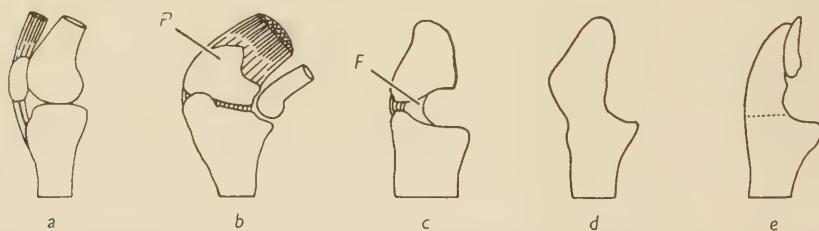
MATERIAL AND METHODS

Dissections have been carried out on preserved wet specimens supplemented by histological examination and inspection of the dried bones where necessary. Developing traction epiphyses have been studied in a number of embryonic and immature limbs.

The following sesamoids have been examined in adult limbs: the patella, the parafibula, those at the elbow joint in some birds, the sesamoid sometimes found in the tendon of plantaris, and the sesamoid in the common extensor origin at the elbow of a tree-shrew, *Tupaia tana*, and that of a bat, *Rousettus aegyptiacus*. Traction epiphyses examined include the tibial crest of some birds, the fibular process present in a few mammals, the great trochanter of the femur, the greater tuberosity of the humerus and the flake epiphysis of the mammalian calcaneus.

FINDINGS

In mammals the patella is typically freely movable and quite widely separated from the tibia (Text-fig. 2a). Fusion of patella and tibia occurs only as an anomaly in mammals, but in the marsupial mole, *Notoryctes typhlops*, there is close apposition between patella and tibia, with an extensive area of fibrous union, and little independent movement is possible (Text-fig. 2b). The avian patella shows more variation. Typically, a patella is present, joined to the tibia by a patellar ligament (e.g. the common cormorant, *Phalacrocorax carbo*, Text-fig. 2c). In some birds the



Text-fig. 2. *a*, typical relationship of patella to the mammalian knee-joint; *b*, position of patella (*P*) in the marsupial mole; *c*, the patella of the common cormorant (*F* is a pad of fibro-fatty tissue); *d*, the tibia, with its crest, in the diving petrel; *e*, tibia with its crest (epiphyseal line dotted) and small proximal patella, in the little grebe.

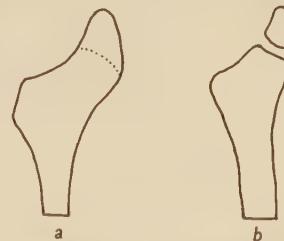
patella is absent but a large bony process, the tibial crest, extends proximally from the front of the tibial articular surface (e.g. the common diving petrel, *Pelecanoides urinatrix*, Text-fig. 2d). As Thompson (1890) suggested, this bears a striking resemblance to a fused patella. In other birds there is a small proximal sesamoid, best termed the proximal patella, as well as the tibial crest (e.g. the little grebe, *Podiceps ruficollis*, Text-fig. 2e). The tibial crest of another diving petrel, *Pelecanoides exsul*, proved to be a muscular process that develops from a separate centre (Pl. 1, fig. 5), thus fulfilling the criteria of a traction epiphysis. The epiphysis and shaft are separated by a fibrocartilaginous plate which becomes ossified in the adult.

In certain mammals, e.g. the spiny ant-eater, *Tachyglossus aculeata* (Text-fig. 3a), the proximal end of the fibula bears a long muscular process. In many others, e.g. the brush-tailed possum, *Trichosurus vulpecula*, the proximal end of the fibula articulates with a large sesamoid, the parafibula (Text-fig. 3b). In at least one species, the wombat, *Phascolomys mitchelli*, there is either a mobile parafibula or one that is fused to the proximal end of the fibula as a muscular process; the separate form was present in six out of seven dried specimens examined and it has also been reported by Pearson & Davin (1921 b). The free parafibula appears, in wet specimens, as a sesamoid in the origin of the lateral head of gastrocnemius or as a compound sesamoid—representing fused cyamella (the sesamoid of the tendon of popliteus) and lateral fabella—in the origins of popliteus and gastrocnemius. The prolongation of the fibula seen in the spiny ant-eater is a traction epiphysis, for the epiphyseal line was clearly visible in the dried skeleton of an immature animal (Text-fig. 3a).

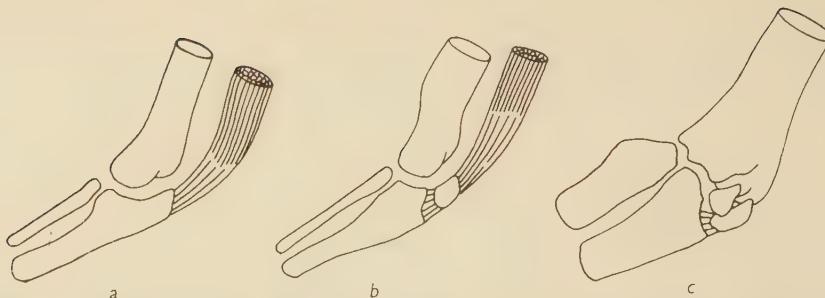
The relationship of the tendons of plantaris, gastrocnemius and soleus to the calcaneus varies in different mammals. In the dog the tendon of gastrocnemius is inserted into the posterior aspect of the calcaneus a short way below its upper margin (Pl. 1, fig. 2), but the plantaris, after winding around the medial aspect of the tendo Achillis, passes superficial to the gastrocnemius attachment to the calcaneus to enter the sole. Here it runs forwards to be inserted into the phalanges in a manner corresponding to the insertions of the plantar fascia and the flexor digitorum brevis muscle of human anatomy. Where the plantaris tendon passes over the posterior aspect of the calcaneus it has a bony-hard sesamoid on its deep surface (Pl. 1, fig. 2). Histological examination showed this to consist of an unusual form of fibrocartilage, as described by Retterer & Lelièvre (1911). In the dog there is another component of the tendo Achillis—a tendon derived from the hamstring muscles, passing down in front of the tendon of gastrocnemius to be attached to the upper margin of the posterior aspect of the calcaneus. This is the tibial aponeurosis (Chauveau, 1891); part of it may represent the absent soleus muscle. In the puppy a flake epiphysis is visible on the posterior aspect of the calcaneus, deep to the sesamoid in the plantaris tendon.

Many birds—e.g. the raven, *Corvus corax* (Text-fig. 4a)—possess an olecranon process on the ulna similar to that of the mammal. In the swift, *Micropus apus* (Text-fig. 4b), however, the ulna lacks an olecranon process but there is a sesamoid in the extensor tendon, which, as Owen (1836) stated, appears to be the missing process. The emperor penguin, *Aptenodytes forsteri*, also lacks an olecranon and the ulna shows a notch where it would be expected. There are two sesamoids at the elbow (Text-fig. 4c), articulating with the lower extremity of the humerus.

Le Gros Clark (1926) has noted a sesamoid in the common extensor origin in the pen-tailed tree-shrew, *Ptilocercus lowii*. A dry specimen of another tree-shrew, *Tupaia tana*, examined by the authors showed this sesamoid to be strikingly similar



Text-fig. 3. a, the fibula, with its crest(epiphyseal line dotted) in the spiny ant-eater; b, fibula and parafibular sesamoid in the brush-tailed possum.



Text-fig. 4. *a*, the elbow of the raven showing olecranon process; *b*, elbow of the swift showing ulnar patella in place of olecranon process; *c*, elbow of the emperor penguin.

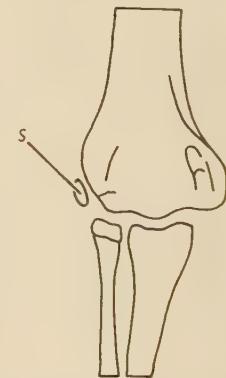
in form to an epiphysis of the lateral epicondyle (Text-fig. 5). A somewhat similar sesamoid was found in a bat, *Rousettus aegyptiacus*.

In the Macropodidae, the greater trochanter has an appearance very suggestive of former independence from the shaft. In an osteoligamentous specimen taken from an immature wallaby (? black-tailed wallaby, *Wallabia bicolor*), the greater trochanter appears as a bony mass set in the course of the tendinous attachment of the gluteal muscles to the shaft and has only a relatively restricted connexion by its deep surface with the junction of head and shaft (Pl. 1, fig. 3). A histological section of a similar stage shows that chondrification has occurred in the central part of the tendinous connexion between greater trochanter and shaft (Pl. 1, fig. 4). Completion of ossification in this region, by extension into this cartilage and its thick tendinous investment, gives rise to the deep trochanteric fossa present in adult macropods. Jenkins (cited by Parsons, 1908) described fibrous tissue intervening between the cartilaginous greater trochanter and the shaft in a human embryo of 18 mm. c.r. length. This could not be seen in 15 and 19 mm. human embryos examined by the authors, but there was some indication of independent chondrification in a 25 mm. human embryo.

No animals are known in which the greater tuberosity of the humerus is represented by a sesamoid. However, in two human embryos of 15 and 25 mm. c.r. length the greater tuberosity appeared to be chondrifying independently (Pl. 1 figs. 6, 7).

DISCUSSION

Two questions are at issue: can it be argued that most traction epiphyses are homologous with pre-existing sesamoids? If so, have traction epiphyses given rise to sesamoids or vice versa? The present findings are in accord with the theory advanced by Parsons that most traction epiphyses are derived from pre-existing sesamoids. The evidence may be summarized under three headings.



Text-fig. 5. Elbow of tree-shrew showing sesamoid (*S*) in position of lateralepicondyle.

Comparative anatomy

The resemblance between the freely mobile patella of some birds and the tibial crest of others is strongly indicative that one structure has given rise to the other. Although some birds (e.g. the grebes) have a proximal patella in addition to the tibial crest, this does not invalidate the view that this crest represents a fused patella, for multiple patellae are known to occur in many mammals—e.g. some of the Felidae, Lagomorpha and Rodentia (Pearson & Davin, 1921a)—and some birds—e.g. the red-crested merganser, *Mergus serrator* (Shufeldt, 1884).

The fact that the patella is separate in all mammals—even the marsupial mole which shows a very extensive tibio-patellar contact—and in most birds supports the contention that the primitive condition is that of a separate sesamoid. It is certainly a weak link in Pearson and Davin's reasoning that they were unable to find a fused patella in any reptile, living or fossil, if the fused condition had, in fact, been the ancestral pattern. Their alternative suggestion that the patella may have been derived from a lunula (i.e. a bony nodule in one of the semilunar cartilages) is almost certainly groundless, for lunulae are intra-articular structures unassociated with tendons (Haines, 1942a; Barnett, 1954).

There is good evidence that the parafibular sesamoid and the fibular process are homologous structures (Pearson & Davin, 1921b). The occasional existence in the wombat of a fused parafibula (i.e. a fibular process) instead of the more usual free parafibular sesamoid strongly supports this contention.

Both the monotremes possess large fibular processes whereas many of the marsupials have a large mobile sesamoid in the corresponding position, and this fact was advanced by Pearson & Davin in support of their theory that sesamoids were the debris resulting from the breaking off of bony processes. However, this does not disprove Parsons's theory, for there is no taxonomic reason why the fibula of a monotreme should not be more specialized, and thus less like the ancestral form, than the marsupial fibula. Again, the fact that no reptile is known with a fibular crest, though some—e.g. *Varanus niloticus*—possess a free parafibular sesamoid (Pearson & Davin, 1921b) indicates that the evolutionary process has been one of fusion rather than separation.

It is probable that the fusion of sesamoid structures in the successive layers of tendons passing over the back of the calcaneus has given rise to the posterior calcaneal epiphysis, leaving the distal parts of these tendons as intrinsic structures of the sole. Thus, the long plantar ligament is generally recognized as the original continuation of the gastrocnemius while the plantar aponeurosis is the tendon of insertion of plantaris (Parsons, 1898; Keith, 1948). Probably the epiphysis on the dog's calcaneus represents a former sesamoid in the gastrocnemius tendon (and perhaps in the tibial aponeurosis). The epiphysis in man would then represent the further incorporation of the sesamoid in the plantaris tendon on the surface of this. Occasionally, radiographs of immature human bones are suggestive of such a lamellated origin of the calcaneal epiphysis (Pl. 1, fig. 1). It is well known that this epiphysis may have multiple centres of ossification (Shanks & Kerley, 1950).

The olecranon process of a bird like the raven has probably arisen by fusion of a sesamoid such as that found in the elbow extensor tendon of the swift—which

lacks an olecranon. Again, the distal sesamoid at the elbow of the emperor penguin, articulating with the trochlea of the humerus, is very reminiscent of an olecranon process. The proximal sesamoid is probably a homologue of the ulnar patella found in many lizards and birds (Haines, 1940; Parsons, 1904).

The free sesamoid in the common extensor origin at the elbow of the tree-shrew, *Tupaia tana*, is remarkably similar both in form and position to the epiphysis usually found for the lateral epicondyle in other mammals. Parsons (1904) noted a similar sesamoid in the bat *Chiromelus torquatus*, and it is present in the bat *Rousettus aegyptiacus*. Proof of the homology between these sesamoids and the epiphysis for the lateral epicondyle in other mammals would require the examination of immature forms.

In many animals, the tendon of the long head of the biceps femoris muscle arises from the vertebral column and the crest of the ilium, and it is often considered that the human sacrotuberous ligament is the proximal degenerate portion of this (Testut, 1904). Bland-Sutton (1902) reported an anomalous human biceps femoris muscle that had a sacral origin only. The tendon contained a sesamoid where it glided over the ischial tuberosity. It may be that the normal ischial traction epiphysis represents such a sesamoid that has become incorporated into the ischium, with the transference of the origin of the biceps to that bone.

A similar anomalous sesamoid has been described in the reflected, or primitive, head of the rectus femoris muscle (McGregor, 1946). Fusion of such a sesamoid to the ilium may have given rise to the anterior inferior iliac spine, a traction epiphysis.

Embryological evidence

The present studies have shown that the fibular process of the spiny ant-eater and the tibial crest of the diving petrel ossify independently of the fibula and tibia, respectively. Pearson & Davin (1921b) have produced some evidence that the fibular process of the platypus, *Ornithorhynchus anatinus*, also ossifies independently. This mode of development is easy to explain if the traction epiphysis had originally been separate, but it is extremely hard to reconcile with Pearson & Davin's theory of fragmentation of a parent bone. One would be obliged to postulate a fortuitous development of a secondary centre in just that part of a bone destined to separate off, thus foreshadowing a state not yet attained in the adult. The mode of development of the great trochanter in the Macropodidae is very suggestive of its origin from an independent sesamoid. The fact that the greater tuberosity of the human humerus (and probably the greater trochanter) shows evidence of independence during the stages of both chondrification and ossification is also in favour of this view.

The histological structure of the developing and adult tibial tubercle and surrounding tissues has been described by Bidder (1906) and Lacroix (1949) in the rabbit and by McLean & Bloom (1940) in the rat. Lewis (1958) has described the structure in man and has suggested that the appearance of the tibial tubercle *anlage*, set in the course of a tendon on its way to be attached to the diaphysis, is consistent with an origin from a sesamoid. Haines (1942b), also, has stressed that many secondary centres of ossification are truly intratendinous. The restricted connexion between the tibia and the intratendinous centre at the knee (the tibial

tubercler) in the lizard *Uromastix spinifer* is certainly indicative of derivation from a pre-existing sesamoid (Lewis, 1958).

Comparison with other evolutionary processes

There is no doubt that bones have frequently joined together during the course of evolution, but there are few instances of one bone 'breaking up' to give rise to two. It is difficult, indeed, to visualize the process by which a free sesamoid could be derived from a bony process to which a tendon is attached, for the separated process would no longer serve as a stable attachment for the tendon. Pearson & Davin's reasoning in this connexion, with regard to the evolution of the parafibula, is unconvincing. On the other hand, fusion of an intratendinous parafibula to the proximal end of the fibula would simply lead to the transference of the origin of the tendon into the new traction epiphysis (the fibular crest), the proximal part of the tendon becoming a ligament joining its old and new attachments. Similar reasoning can explain the origin of the calcaneal epiphysis and the epiphysis of the ischial tuberosity.

The evidence in favour of the origin of traction epiphyses from sesamoids is of the same order as that taken to prove the threefold nature of the human innominate bone. This evolutionary origin is never questioned, yet the evidence for it is indirect, based upon embryology and comparative anatomy. The influence of terminology is apparent here: it is probable that if the bone forming the greater trochanter of the femur were given an independent name, its identity as a separate structure would not be questioned any more than are the separate identities of the ilium, ischium and pubis.

It is necessary, finally, to discuss why the theory advanced by Parsons has received little support. Haines's (1940) main objection to the theory was based on Parsons's interpretation of the reptilian elbow. Parsons described an ulnar patella in the tendon of triceps in many lizards and noted that at the elbow of the young *Sphenodon* there was apparently a similar sesamoid which joined the upper end of the ulna in the adult. He maintained that this was an example of a sesamoid in a young animal being converted to a traction epiphysis in the adult.

Haines (1940) pointed out that two ossific centres are developed in the triceps tendon of most reptiles—a proximal one (the ulnar patella), which remains free, and a distal one connected to the ulna by cartilage in the young and fusing with it in the adult. *Sphenodon*, however, has no ulnar patella; it has a single bony nodule corresponding to the distal ossific centre of most reptiles. Thus Parsons had really described the fusion of a distal centre and not of an ulnar patella. Haines certainly demonstrated that Parsons had erred in citing the development of *Sphenodon* in support of his theory of fusion, but he did not, as is often assumed, disprove Parsons's general theory. Haines did not, in fact, consider the possibility that the distal centre may itself be evolved from a pre-existing sesamoid (see d, Text-fig. 1).

The reason why Pearson & Davin refused to countenance the theory that sesamoids could have given rise to traction epiphyses requires mention. In their opinion, the view that a sesamoid could arise in a tendon 'if further strength were required', demands the 'acceptance of Lamarekism to ensure its perpetuation'. It is, however, equally difficult to imagine the process by which natural selection

could give rise to a sesamoid bone, in an advantageous position, unless all tendons can be shown customarily to exhibit small nodules of cartilage or bone in random positions along their length. It seems unreasonable to ignore the probable derivation of many traction epiphyses from sesamoids merely because the mechanism of origin of sesamoids in the first instance cannot readily be explained in terms of current evolutionary theories.

It is likely that sesamoids arise in tendons as a result of pressure, rather than as bony debris resulting from the break-up of bony processes. Their perpetuation may be ensured by some such mechanism as has recently been proposed by Waddington (1957) for the selective incorporation of acquired characters into the genetical make-up of a species.

SUMMARY

1. A number of sesamoids commonly present at the knee, elbow and heel regions in mammals and birds have been examined.
2. The occurrence in some animals of traction epiphyses corresponding in form and position to these sesamoids indicates that one type of structure has probably given rise to the other.
3. The developmental stages of certain traction epiphyses have been studied. The findings suggest that they were formerly independent of the bones to which they now belong.
4. The evidence is discussed in favour of Parsons's theory that many traction epiphyses have evolved from pre-existing sesamoids.

We are indebted to Prof. D. V. Davies for the provision of material and for criticizing the manuscript, Dr J. D. MacDonald of the British Museum (Natural History) for the supply of avian material, Mr A. V. Freeborn for technical assistance and Mr A. L. Wooding for the photographs.

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EXPLANATION OF PLATE

Fig. 1. Radiograph showing a laminated calcaneal epiphysis (human).

Fig. 2. Dissected leg of a dog showing the sesamoid (*S*) in the tendon of plantaris.

Fig. 3. Great trochanter region of an immature wallaby (? black-tailed); white card in trochanteric fossa.

Fig. 4. Histological section through the great trochanter region of an immature black-tailed wallaby ($\times 4\frac{1}{2}$).

Fig. 5. Upper end of tibia of immature diving petrel showing the temporary fibro-cartilaginous septum between the tibial crest and shaft ($\times 6$).

Fig. 6. Coronal section through the greater tuberosity region of a human embryo of 25 mm. c.r. length showing partial independence of the tuberosity (*T*) ($\times 41$).

Fig. 7. Transverse section through great tuberosity region of a human embryo of 15 mm. c.r. length showing evidence of independent chondrification of the greater tuberosity (*T*) ($\times 104$).

THE TENDONS OF FLEXOR DIGITORUM PROFUNDUS

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INTRODUCTION

Although the arrangement of the flexor digitorum sublimis* tendons in the hand is described in detail in most anatomical works, surprisingly little comment has been made upon the form of the flexor digitorum profundus tendons. In many accounts the actual shape of the profundus tendons is not mentioned at all (Wood Jones, 1944; Grant, 1948; Wood Jones, 1949; Lockhart, 1951; Johnston & Whillis, 1954; Last, 1954), though a few authors state that the profundus tendon is rounded where it passes between the two divisions of the sublimis (e.g. Bryce, 1923; Grant & Smith, 1953). Henle (1871) and Poirier & Rouvière (1912), however, noted that the profundus tendon is narrowed and cylindrical where it lies in the slit in the sublimis tendon, but that as soon as it has passed through the slit, it becomes wider and flat, and develops a median longitudinal furrow on its palmar aspect. Poirier & Rouvière (1912) stated that the fibre bundles of the distal part of the tendon turn towards, and sink into this median furrow. Henle (1871) alone noted that the tendon again becomes narrowed opposite the terminal interphalangeal joint, after which it expands fan-wise before its insertion into the base of the terminal phalanx.

The present study has shown that the profundus tendon is accurately moulded to conform to the varying outline of the sublimis tunnel, though it is oval prior to entering and also after leaving the tunnel, and that this moulding of the tendon is brought about by a rearrangement of its constituent fibre bundles.

The flexor digitorum longus tendons in the foot undergo a similar change of shape when passing through the flexor digitorum brevis tendons.

MATERIALS AND METHODS

Twenty pairs of flexor digitorum profundus and flexor digitorum sublimis tendons were removed from the hands of dissecting-room cadavers, and ten pairs from post-mortem room cadavers. The tendons were divided in the mid-palm and detached from their insertions into the phalanges. After examination of the profundus and sublimis tendons in their normal relationship, most of the post-mortem specimens of profundus tendons were separated from the sublimis tendons and pinned out on cork. After the fresh tendons had been examined macroscopically, they were fixed either in 10 % formalin or in Heidenhain's 'Susa'. Some of the fixed tendons were embedded in paraffin, and others in celloidin. From a celloidin block containing a profundus and a sublimis tendon in their normal relationship, transverse sections were cut free-hand, using a small microtome knife: these sections were stained with

* The International Anatomical Nomenclature Committee (1955) have suggested that the name of this muscle be changed to the flexor digitorum superficialis.

haematoxylin and eosin and mounted on slides. From paraffin and celloidin blocks containing only profundus tendons, longitudinal sections were cut at 30μ with a sledge microtome; some of these sections were stained with haematoxylin and eosin and others with van Gieson. Somewhat better sections were obtained from celloidin than from paraffin blocks, but even in these there was a tendency for the tendon fibres to tear and pull out of the block, even though the cut surface of the block was coated with a layer of celloidin prior to cutting each section.

The flexor digitorum sublimis and profundus tendons of two 6-month-old foetuses were also embedded in paraffin, sectioned transversely at 20μ , and stained with haematoxylin and eosin.

Information on the fibre bundle arrangement of the profundus tendon was obtained by macroscopic and microscopic examination, and also by carefully shredding some of the dissecting-room specimens with fine forceps.

Six pairs of flexor digitorum brevis and flexor digitorum longus tendons were obtained from dissecting-room specimens, and two pairs from a freshly amputated foot. These tendons were only examined macroscopically: the flexor digitorum longus tendons showed the same change of form as the flexor digitorum profundus tendons.

RESULTS

In the mid-palm, each tendon of flexor digitorum sublimis is oval in shape, but as it crosses the base of the first phalanx of the finger, it becomes flattened and then divides into two halves: each flattened half of the tendon passes downwards and backwards around the side of the profundus tendon to gain its dorsal aspect, and in doing so undergoes a twist whereby the fibre bundles which were originally lateral are brought to a medial position where they decussate with the corresponding fibres of the opposite half of the tendon—the chiasma tendinosum of Camper (*Ch* in Text-fig. 1B). The fibres which were originally medial in position finally take up a lateral position and do not decussate. In the region of the decussation there is a thick band of synovial membrane (*Sy* in Text-fig. 1B) which is connected to the profundus tendon by a tag or vinculum from each of its free margins (*V1*, *V2* in Text-fig. 1B): these vincula must be severed in order to separate the tendons.

The flexor digitorum profundus tendon is narrowed from side to side where it passes between the diverging halves of the sublimis tendon (*A* in Text-fig. 1C), and is somewhat deepened antero-posteriorly (*A* in Text-fig. 1F; *A* in Pl. 1, fig. 5). In this narrowed portion, the sides of the tendon are flattened, and shelve in such a way that they are adapted to the flat diverging halves of the sublimis tendon, with the result that the palmar aspect of the tendon in this region is V-shaped (*V* in Text-fig. 1C): the apex of the V is directed proximally and corresponds to the point of division of the sublimis tendon. The succeeding portion of the tendon (*B* in Text-fig. 1C) lies in the gutter formed by the two halves of the sublimis tendon as they leave its sides and gain its dorsal aspect. This portion of the profundus tendon is adapted to the gutter and is therefore broad on its palmar aspect and narrow on its dorsal aspect, which is the reverse shape to that of its preceding segment. Just before it leaves the sublimis gutter, the tendon again becomes oval in shape, and

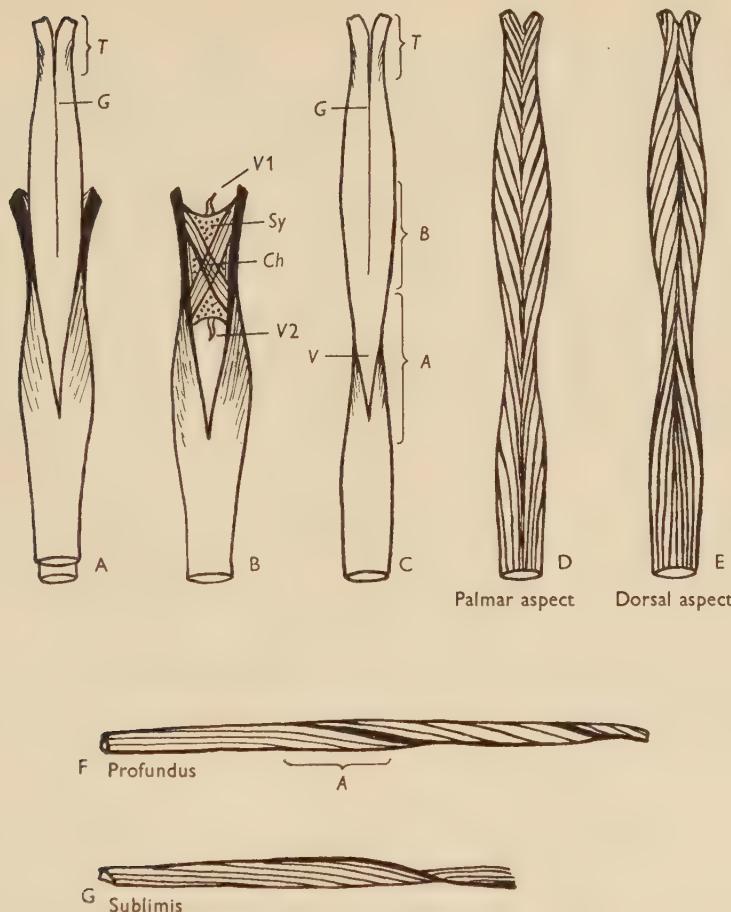
develops on its palmar aspect a median longitudinal groove, which gradually deepens as it is traced distally (*G* in Text-fig. 1A, C). The tendon terminates in a fan-shaped expansion, but before doing so it undergoes a second narrowing from side to side (*T* in Text-fig. 1A, C), and becomes almost completely subdivided by the palmar median groove (Pl. 1, figs. 19, 20).

The changes in shape of the profundus tendon and their relationship to the changes in outline of the sublimis tunnel can be clearly followed in transverse sections (Pl. 1, figs. 6–20). Prior to the division of the sublimis tendon, both sublimis and profundus tendons are oval in shape (Pl. 1, figs. 6, 7). Just after the sublimis divides, its two flattened halves form an inverted V, and the profundus tendon is here triangular in outline, with the apex of the triangle on its palmar aspect (Pl. 1, figs. 9, 10). As the two halves pass dorsally along the sides of the profundus, they diverge from one another, and here the outline of the profundus tendon gradually changes from triangular to trapezoid, with the palmar border narrower than the dorsal (Pl. 1, figs. 11, 12). However, after the two halves of the sublimis have turned around the margins of the profundus to gain its dorsal aspect, they form a segment of an oval, and here the profundus tendon becomes almost square in outline (Pl. 1, fig. 13). After gaining the dorsal aspect of the profundus, the two halves of the sublimis form a gutter which is at first V-shaped, but the two limbs of the V gradually open out so that the gutter becomes progressively shallower (Pl. 1, figs. 14–17), and the sublimis then terminates by dividing into two bundles (Pl. 1, fig. 18). The segment of the profundus tendon occupying this gutter (i.e. segment *B* in Text-fig. 1C) undergoes the reverse series of changes in outline to those described above for the preceding segment (i.e. segment *A* in Text-fig. 1C). Thus its outline first changes gradually from being almost square (Pl. 1, fig. 13) to trapezoid, with the palmar border broader than the dorsal (Pl. 1, figs. 14, 15), then to triangular (Pl. 1, fig. 16), and finally it becomes oval (Pl. 1, figs. 17, 18). Near its distal extremity the tendon becomes narrowed from side to side and is then almost completely subdivided by the palmar median groove (Pl. 1, figs. 19, 20).

The same series of changes in outline of the profundus tendon occurs in the foetal tendon (Pl. 1, figs. 21–26).

It was found that the changes in shape of the profundus tendon are brought about by changes in direction of its constituent fibre bundles. On macroscopic examination (Text-fig. 1 D, E), it can be seen that before the tendon enters the sublimis tunnel the superficial palmar fibre bundles run parallel to the long axis of the tendon, but just before the tendon passes between the two halves of the sublimis, these fibre bundles diverge from the mid-line of the tendon and pass obliquely downwards to its sides (Text-fig. 1 D). After curving around the sides of the tendon to gain its dorsal aspect, the fibre bundles reverse their direction and run obliquely downwards towards its mid-dorsal line (Text-fig. 1 E). Thus, the fibres of this part of the profundus tendon undergo a spiral turn, similar to that undergone by the fibres of the sublimis tendon, whereby fibres which were originally lateral in position become medial, a point which can be appreciated on examination of the two tendons from their sides (Text-fig. 1 F, G).

The behaviour of the fibre bundles at the mid-line of the palmar and dorsal aspects of the profundus tendon was studied in shredded tendons. It was found that near



Text-fig. 1. Key. *A*, segment of profundus tendon that passes between the two halves of the sublimis tendon; *B*, segment of profundus tendon that occupies the sublimis gutter; *Ch*, chiasma tendinosum; *G*, median groove on palmar aspect of profundus tendon; *Sy*, band of synovial membrane; *T*, terminal part of profundus tendon; *V*, the V-shaped area on palmar aspect of profundus tendon; *V1*, *V2*, vincula tendinum.

(A) Tendons of flexor digitorum sublimis and profundus in their normal relationship; palmar aspect. Note the narrowing of the terminal part of the profundus tendon just before it is subdivided by the median palmar groove. (B) Flexor digitorum sublimis tendon; palmar aspect. After division of the tendon, each flattened half twists so that fibres originally lateral become medial and decussate with those of the opposite side. Note synovial band and its vincula in region of decussation. (C) Flexor digitorum profundus tendon; palmar aspect. Segment *A* lies between the diverging halves of sublimis; it is narrowed from side to side and its palmar aspect is V-shaped. Segment *B* lies in the sublimis gutter; it is broad on its palmar aspect and narrow on its dorsal aspect. (D, E) Flexor digitorum profundus tendon; fibre bundle arrangement as seen from palmar and dorsal aspects respectively. The superficial palmar fibre bundles diverge from the mid-line to the sides of the tendon, gain its dorsal aspect and then pass to the mid-dorsal line, thereby undergoing a spiral turn. (F) Flexor digitorum profundus tendon; side aspect. Note that the superficial fibre bundles undergo a spiral turn in passing from palmar to dorsal aspect of the tendon. (G) Flexor digitorum sublimis tendon; side aspect of one half. Note that the fibre bundles undergo a spiral turn.

the apex of the V on the palmar aspect (Text-fig. 1C), fibres from the deeper part of the tendon start emerging at its mid-line to become superficial, and the process is continued to the distal end of the tendon; these fibres diverge from the mid-line and gain the dorsal aspect of the tendon in the same manner as those preceding them (Text-fig. 1D). This is a necessary arrangement to make good the loss of superficial fibres which have already passed from the palmar to the dorsal aspect of the tendon. On reaching the mid-line of the dorsal aspect of the tendon, the fibre bundles enter the substance of the tendon; successive fibre bundles, on reaching the mid-line, overlie superficially those immediately preceding them (Text-fig. 1E). The result of this arrangement is that the deep fibre bundles in the central part of the tendon take an oblique course as they pass distally, namely, from the dorsal to the palmar aspect of the tendon, a feature which can be seen in longitudinal sections taken from near the centre of tendons that have been cut antero-posteriorly (Pl. 1, fig. 5).

Longitudinal sections of the profundus tendon cut in the plane of the palm illustrate the change of shape which the tendon undergoes as it passes through the sublimis tunnel, and also the change in direction of the fibre bundles of the tendon. In sections taken from near the palmar surface of the tendon (Pl. 1, figs. 1, 3), the marked narrowing which the tendon undergoes where it is embraced by the sublimis just after the latter divides can be seen, as well as the marked obliquity of the fibre bundles proximal to the narrowed portion. Sections near the central part of the tendon (Pl. 1, figs. 2, 4) show that the narrowing is less marked here because the two halves of the sublimis tendon in apposition to it are now farther apart: although the fibre bundles at the periphery of the latter sections are oblique in direction, those of the central part are not.

DISCUSSION

Although the mode of formation of the flexor digitorum sublimis tunnel is well understood, it has not previously been made clear that the outline of the tunnel varies throughout its length. The two halves of the sublimis, just after its division, form two sides of a triangle with the apex directed anteriorly. As the two halves pass to the sides of the profundus tendon they form part of the outline of an oval; after gaining the dorsal aspect of the profundus, they again form two sides of a triangle for a short distance, with the apex directed posteriorly, and when they decussate, they form a shallow gutter, the outline of which is a segment of an oval.

It is therefore not surprising to find that the segment of the profundus tendon which passes through the sublimis tunnel varies in shape, whereby its outline conforms to that of the tunnel.

It is probable that during the normal movements of flexion and extension of the fingers, very little movement takes place between the sublimis and profundus tendons, since movements at the proximal interphalangeal joint normally take place concomitantly with those at the distal interphalangeal joint. In any case, the vincula uniting the two tendons do not allow of very much movement between the tendons. It would appear therefore that during movements of the finger, the specially moulded segment of the profundus tendon does not shift its position within the sublimis tunnel to any great extent.

SUMMARY

1. It has been found that whereas each tendon of the flexor digitorum profundus is oval in shape prior to entering and also after leaving the tunnel formed by the division and partial reunion of the corresponding tendon of the flexor digitorum sublimis, the segment of the profundus tendon which occupies the sublimis tunnel is so moulded that its outline conforms to the varying shape of the tunnel. A similar moulding is present in the flexor digitorum longus tendons in the foot where they pass through the tendons of the flexor digitorum brevis.

2. The localized moulding of the profundus tendon is brought about by a change in direction of its fibre bundles. In the region of the moulding, the palmar fibre bundles deviate from the mid-palmar line of the tendon to its sides, gain its dorsal aspect and then pass to the mid-dorsal line; thus they pursue a spiral course similar to that undergone by the fibre bundles of the sublimis tendon. The fibre bundle arrangement can be seen on macroscopic and on microscopic examination, and can be demonstrated by shredding the tendon.

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EXPLANATION OF PLATE

Fig. 1. Flexor digitorum profundus tendon; L.S. cut in the plane of the palm from near the palmar surface of the tendon. H. and E. The tendon is markedly narrowed where it is embraced by the sublimis tendon, just after the latter divides. Note the obliquity of the fibre bundles.

Fig. 2. Flexor digitorum profundus tendon; L.S. from near the centre of the same tendon as in fig. 1. The narrowing of the tendon is less marked here since the halves of the sublimis in apposition to it are farther apart. Note the obliquity of the fibre bundles.

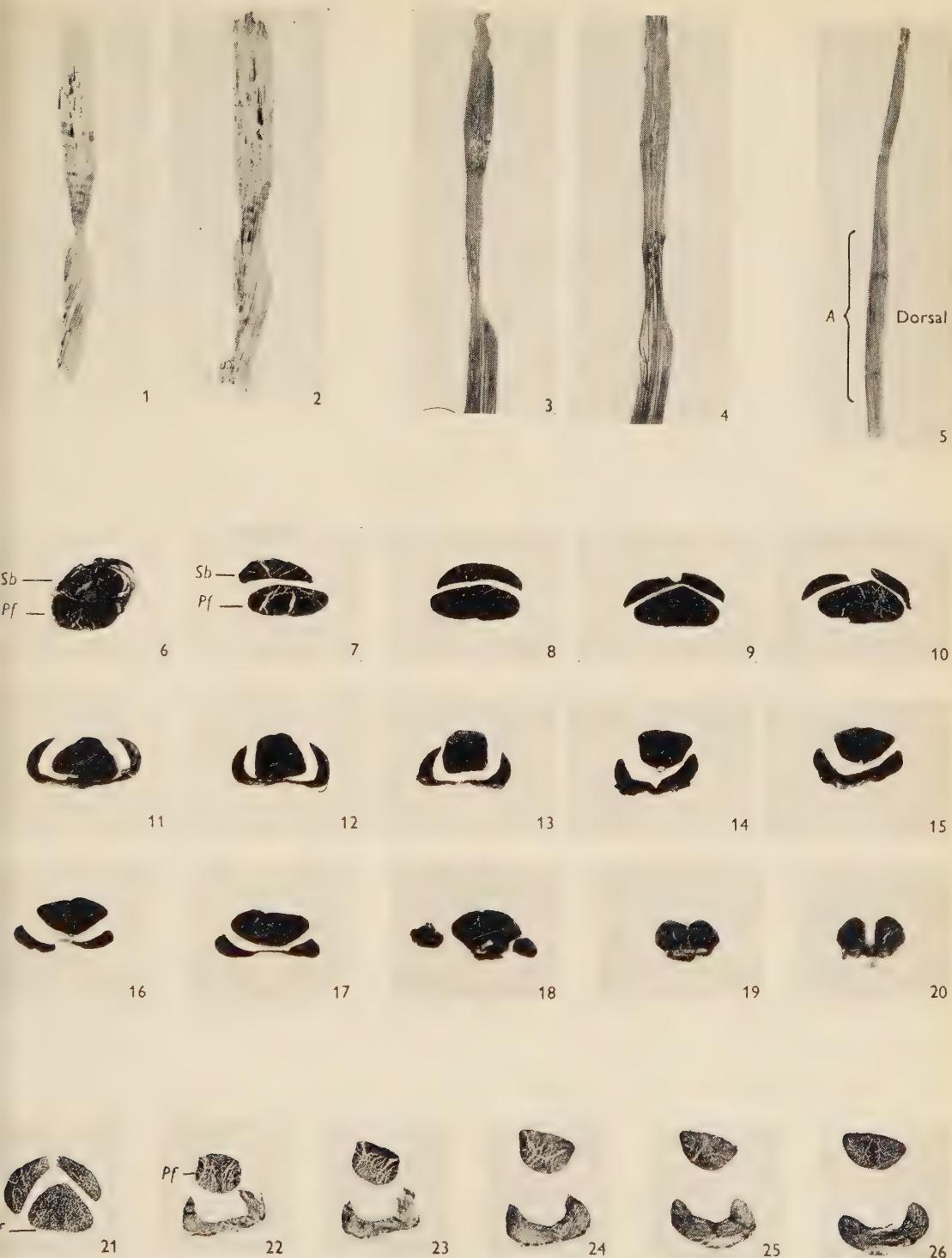
Fig. 3. Flexor digitorum profundus tendon; L.S. cut in the plane of the palm from near the palmar surface of the tendon. H. and E. The plane of section is somewhat different from that of tendon in fig. 1, but the marked narrowing of the tendon as well as the obliquity of the fibre bundles can be seen.

Fig. 4. Flexor digitorum profundus tendon; section from near centre of same tendon as in fig. 3. Here the tendon is less markedly narrowed and the fibre bundles of its central part do not deviate.

Fig. 5. Flexor digitorum profundus: A.P. section from near the centre of the tendon. H. and E. The segment which passes between the diverging halves of the sublimis tendon (segment *A*) is deeper antero-posteriorly than the more distal segment; the direction of the fibre bundles as they are traced distally is from the dorsal to the palmar aspect of the tendon.

Figs. 6–20. Representative transverse sections of a sublimis and a profundus tendon, arranged in series from the mid-palm onwards. H. and E. $\times 2$. In the mid-palm, both tendons are oval and are invested with the common synovial flexor sheath (fig. 6). As the profundus tendon (*Pf*) passes between the two divisions of the sublimis (*Sb*), its outline changes from oval to triangular, then to trapezoid, and then becomes almost square (figs. 6–13): as it lies in the sublimis gutter, its outline changes from square to trapezoid, then to triangular, and then to oval (figs. 13–18); its terminal portion is subdivided by the median palmar groove (figs. 19, 20).

Figs. 21–26. Representative transverse sections of a sublimis and a profundus tendon from a six-months-old foetus. H. and E. $\times 16$. In passing through the sublimis tunnel, the outline of the profundus tendon (*Pf*) becomes triangular, then almost square, then trapezoid, then triangular and finally oval.



MARTIN—THE TENDONS OF FLEXOR DIGITORUM PROFUNDUS

(Facing p. 608)

THE OBLIQUE CORD OF THE FOREARM

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INTRODUCTION

The oblique cord of the forearm (chorda obliqua antebrachii anterior; ligament of Weitbrecht, 1742) is a fibrous cord or band which passes from the lateral side of the tuberosity of the ulna, downwards and laterally to gain attachment to the anterior surface of the radius, just below the radial tuberosity. The cord becomes slack in pronation of the forearm and tense in supination, and may assist in preventing over-supination (Morris, 1879; Fick, 1904; Nicolas, 1926). Morris (1879) believed that it acts as a stay or support to the radius at a point rendered weak by the meeting of two curves on that bone.

Some authors (e.g. Grant, 1952; Walmsley, 1951; Last, 1954) quote the view that the cord may represent a degenerated portion of the flexor pollicis longus. This view of the morphological significance of the cord stems from the observations of Fawcett (1895), Forster (1905) and Rouvière & Granel (1909). Both Fawcett and Rouvière & Granel drew attention to the fact that the flexor pollicis longus commonly receives a head of origin from the lateral side of the ulnar tuberosity. Rouvière & Granel observed this head in sixty-one of eighty-four subjects (composed of sixty adults, four newborn and twenty foetuses), and Fawcett found this head to be seldom absent, whereas a head arising from the medial side of the ulnar tuberosity, described in text-books as the 'occasional' head, he found to be of infrequent occurrence. From their observations that the head of the muscle from the lateral side of the ulnar tuberosity has a similar disposition to the oblique cord, is frequently attached to it near its origin, and occasionally replaces it, Fawcett (1895) and Rouvière & Granel (1909) concluded that the oblique cord represents a degenerated, dismembered part of this head of the flexor pollicis longus. Forster (1905) studied in detail the arrangement of the oblique cord and its relationship to the flexor pollicis longus in forty-two adults and two foetuses, and he also concluded that it represents an accessory bundle of this muscle.

Opposed to this view of the significance of the oblique cord is that put forward by Macalister (1889), who considered the cord to be a strengthened band of the fascia over the supinator muscle; a similar opinion was held by Poirier (quoted by Nicolas, 1926), who regarded it as a condensation of the connective tissue interposed between the biceps tendon and the supinator muscle.

The results of the present investigation support the view of Macalister.

MATERIALS AND METHODS

Two sets of dissected parts were studied. The first set was composed of twenty-one forearms in which the main dissection had already been carried out, leaving the deep portion of the supinator muscle and the joints intact. The second set

comprised twenty-one forearms which were undergoing routine dissection, and in which the muscle arrangements in the forearm could be investigated.

RESULTS

Examination of the twenty-one deep dissections of the forearm revealed the following facts regarding the origin of the deep head of the supinator muscle. In every specimen, the deep head of the muscle arose, not only from the posterior part of the bicipital fossa of the ulna, as commonly described, but from the whole of the fossa, so that the anterior fibres were attached to its well-marked anterior margin, which, as it is traced upwards from the interosseous border of the ulna, inclines forwards towards the lateral side of the coronoid process (Fig. 1 (1)). In some specimens, the upper fibres of this part of the supinator lay anterior to the lower ones, and in two specimens they formed a very thick bundle which was separate from the remaining fibres superiorly but blended with them inferiorly (Fig. 1 (2)). In many of the specimens, however, the anterior part of the deep head of the supinator was fibromuscular. In some of these cases, the anterior surface of this part of the muscle was covered by a fibrous membrane, the upper portion of which was thickened and formed the oblique cord, and fibres of the supinator arose from the deep aspect of the cord and membrane (Fig. 1 (3, 5)). In other cases, fibrous strands and bands were intermingled with the muscle fibres of the anterior part of the muscle, and the oblique cord was derived from the fibrous component; occasionally several fibrous bands accompanied the cord and were attached to the radius just below it (Fig. 1 (4)). The oblique cord varied in form from a rounded cord to a flat membrane, the latter being attached to the anterior margin of the bicipital fossa of the ulna. It was confirmed that the cord becomes slack in pronation and tense in supination of the forearm, which suggests that it is a factor concerned in limiting supination.

In the second series of dissections the muscle attachments in the upper region of the front of the forearm were studied, in addition to the form and arrangement of the oblique cord.

In nine of the twenty-one specimens, an additional head of the flexor pollicis longus was found, which arose from the lateral side of the tuberosity of the ulna and sometimes from the supinator muscle as well, and which blended below with the superior extremity of the flexor pollicis longus. In only four specimens was there an additional head from the medial aspect of the forearm. The latter is the 'occasional' head described in text-books of anatomy, and will be referred to as such, whilst the former will be referred to as the 'additional' head.

The occasional head was fusiform in two cases and in the form of a flat strip in the other two: one of the fusiform heads arose from the medial aspect of the tuberosity of the ulna but in the other three it arose from the common flexor origin. In each case, this head joined the tendon on the medial border of the flexor pollicis longus. In two of these specimens, an additional head of the flexor pollicis longus was present as well as the occasional head (Fig. 1 (7)).

The additional head of the flexor pollicis longus, found in nine specimens, was muscular in some cases, membranous in others, and in the remainder it was membranous above and muscular below (Fig. 1 (7, 8)). The degree of development of

this head varied from specimen to specimen, and, as noted by Forster (1905), even differed between the two limbs of the same cadaver. In every case this head was in the form of a flat strip, the surfaces of which faced medio-laterally.

In nine of the twelve specimens in which the flexor pollicis longus did not possess a distinct additional head, an additional head of flexor digitorum sublimis* was

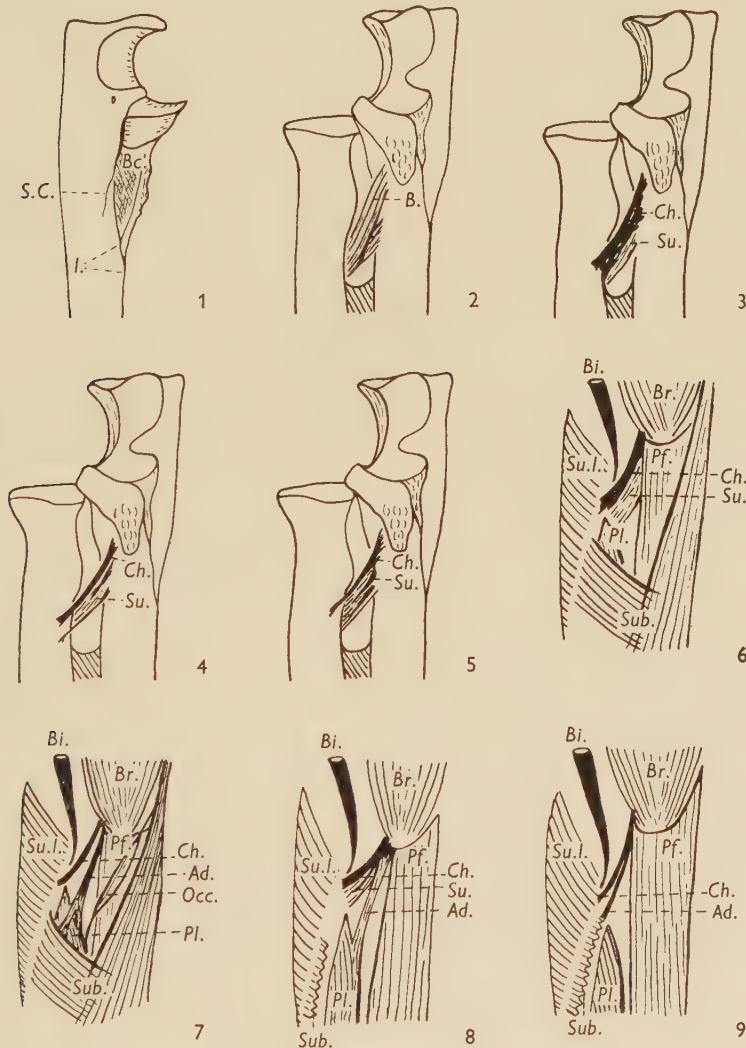


Fig. 1. For explanation see end of paper.

present, and arose from the lateral side of the tuberosity of the ulna and joined the upper margin or posterior aspect of the radial head of the flexor digitorum sublimis, near to its origin from the anterior oblique line of the radius (Fig. 1 (9)). This head

* The International Anatomical Nomenclature Committee (1955) have suggested that the name of this muscle be changed to flexor digitorum superficialis.

was in the form of a membranous band at its origin in all nine cases and its surfaces, like those of the additional head of flexor pollicis longus, faced medio-laterally. Inferiorly, the band sometimes gave way to a rounded bundle of muscle fibres, which, in some instances, gave fibres to the flexor pollicis longus as well as to flexor digitorum sublimis (Fig. 1 (7)). It was not uncommon for the additional heads of flexor pollicis longus and flexor digitorum sublimis to blend with neighbouring structures near their origins, namely, the flexor digitorum profundus, oblique cord and supinator muscle (Fig. 1 (7-9)).

In only three of the twenty-one specimens examined was there no additional head of either the flexor pollicis longus or the flexor digitorum sublimis.

An oblique cord was found in fourteen of the twenty-one specimens, and, as in the first series of dissections, it was flat and membranous in some cases and rounded in others. In all cases it was deep to the additional head of flexor pollicis longus or of the flexor digitorum sublimis. The cord did not have the same direction, however, as that of the overlying muscle head: although it originated from the lateral side of the tuberosity of the ulna, near to the origin of the muscle head, and was often partly blended with it there, it invariably deviated laterally from the muscle head in its downward course to its radial attachment (Fig. 1 (7-9)). In only a few instances was the radial attachment of the cord near to the superior extremity of the main (radial) head of the flexor pollicis longus; in most cases, the point of attachment was situated some distance above the muscle (Fig. 1 (8, 9)). A much closer relationship was found, however, between the cord and the underlying supinator muscle. In five of the fourteen specimens, it was clear that the cord was formed as a thickening in the upper part of a layer of fascia overlying the anterior aspect of the supinator, and was easily separated as a distinct entity from the adjacent, much thinner fascia. In other specimens, the cord was the only obvious fibrous band found in apposition with the supinator; in one case, the cord lay in contact with a distinct bundle of the supinator (Fig. 1 (6), compare with (2)). The direction of the oblique cord was the same as that of the fibres of the supinator, and in many cases fibres of the muscle were attached to the deep aspect of the cord. In nine specimens the radial attachment of the cord was very closely related to the insertion of the supinator, near the upper end of the anterior oblique line of the radius (Fig. 1 (6-9)).

It was noteworthy that where the oblique cord was in the form of a fascial band, the surfaces of the band faced rather more antero-posteriorly than did those of the occasional heads of flexor pollicis longus and flexor digitorum sublimis, which faced almost directly medio-laterally.

DISCUSSION

The observation made by Fawcett (1895), Forster (1905) and Rouvière & Granel (1909), that an additional head of the flexor pollicis longus from the lateral side of the tuberosity of the ulna is commonly present, was confirmed in the present work. As Fawcett (1895) pointed out, this head occurs much more frequently than the head from the medial side of the ulna, yet even to-day it is the latter (the 'occasional' head) which is recorded in text-books of anatomy, to the exclusion of the former. In addition, it was confirmed that not infrequently the radial head of the flexor

digitorum sublimis also receives a head of origin from the lateral side of the tuberosity of the ulna; this head was membranous at its origin in all cases in the present series, but sometimes gave way to muscle fibres below, some of which joined the flexor pollicis longus in a few specimens.

From careful study of the relationship of the oblique cord to the flexor pollicis longus, it is concluded that the cord does not represent a degenerate head of this muscle, as claimed by Fawcett (1895), Forster (1905) and Rouvière & Granel (1909). In the first place, an oblique cord as well as an additional head of the flexor pollicis longus (or of the flexor digitorum sublimis) was present in most specimens examined. The cord may be blended at its origin with nearby muscles, such as the flexor digitorum profundus or the additional heads of flexor pollicis longus and flexor digitorum sublimis, but this, as Forster (1905) pointed out, is probably of no particular significance. It would be of considerable significance, on the other hand, if the distal extremity of the cord joined the main head of flexor pollicis longus with a reasonable degree of consistency, but in the specimens examined this was not the case. In only a few cases was the cord attached very near to the upper extremity of this muscle, but never joined it; in the remainder, the cord was attached above it. Indeed, Rouvière & Granel (1909) also found in their series that the cord is attached to the radius a variable distance (even as much as 1 cm) above the flexor pollicis longus, and again, Forster (1905) in his series of forty-two cases, found that the cord made contact with, or passed directly into the flexor pollicis longus in only eleven cases, and in only two of these was the lower part of the cord muscular. It is quite possible, judging especially from his illustrations, that at least in some instances, Forster was mistaking the additional head of flexor pollicis longus for the oblique cord itself.

The present finding that the oblique cord lies deep to the additional head of flexor pollicis longus, diverges from it laterally in its course distally, and is normally attached to the radius above the main part of the muscle, does not support the view that the cord is a degenerate part of the additional muscle head, unless it is a specialized, dismembered portion, as proposed by Fawcett (1895) and Rouvière & Granel (1909), brought into being by the movements of pronation and supination (Fawcett, 1895), and perhaps by compression of muscle fibres against the tendon of the biceps and the radial tuberosity (Rouvière & Granel, 1909). If, however, the cord is assumed to be a fibrous transformation of a dismembered part of a muscle bundle, there is no reason why it could not be derived from the additional head of flexor digitorum sublimis, which occurs just about as frequently as that of flexor pollicis longus and has almost the same disposition.

It was found in the present study, however, that the oblique cord stands in much closer relationship to the deep head of the supinator muscle than it does to the additional heads of flexor pollicis longus and flexor digitorum sublimis. Although Morris (1879) noted that the proximal part of the cord is closely related to the supinator, this relationship has not been generally appreciated, and the reason may well be that the deep head of the supinator has always been assumed to arise from only the posterior part of the bicipital fossa of the ulna, whereas it was found in the present investigation that the muscle arises from the whole of this fossa. It was found, furthermore, that in some specimens the upper fibres of the deep head of the

supinator form a distinct muscle bundle, which is attached proximally to the side of the ulnar tuberosity but is blended inferiorly with the remainder of the muscle head. In these specimens, the oblique cord was absent or was developed as a fibrous band in close contact with this muscle bundle. In most specimens, however, the cord was developed either as a thickening in a layer of fascia overlying the supinator, or, when the anterior part of the supinator was fibromuscular, it was derived from the fibrous component. When the cord is in the form of a flat membrane, it is attached proximally to the anterior margin of the bicipital fossa of the ulna, as illustrated by Fick (1904), and it is noteworthy that the surfaces of the membrane face rather more antero-posteriorly than do those of the additional heads of flexor pollicis longus and flexor digitorum sublimis, which face medio-laterally. Commonly, fibres of the supinator muscle are attached to the posterior aspect of the oblique cord, but inferiorly the cord loses contact with the muscle to become attached to the anterior surface of the radius, but at its radial attachment it normally comes once again into very close relationship with the supinator, near the insertion of this muscle into the anterior oblique line of the radius, a point also noted by Fick (1904).

It is concluded from this study that the oblique cord is formed as a thickening of the fascia overlying the supinator muscle, as originally proposed by Macalister (1889), or from a fibrous transformation of the deep head of this muscle, and the suggestion is put forward that the cord represents a degenerate part of the supinator muscle, a part which appears in some specimens as a distinct muscle bundle.

SUMMARY

1. It has been confirmed that the flexor pollicis longus frequently has an additional head of origin from the lateral side of the tuberosity of the ulna, and this is present much more frequently than the 'occasional' head from the medial aspect of the tuberosity. An additional head of the flexor digitorum sublimis may also arise from the lateral side of the tuberosity of the ulna.

2. It is concluded from the present study that the oblique cord does not represent a degenerate part of the additional head of flexor pollicis longus, but that it is formed as a thickening of the fascia overlying the supinator, and may represent a degenerate part of the supinator, a part which in some specimens appears as a distinct muscle bundle.

I should like to thank Prof. F. Davies for his helpful criticism in the preparation of the manuscript.

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EXPLANATION OF FIGURE 1

Illustrations 2–5 show the relationship of the oblique cord to the deep head of the supinator muscle; illustrations 6–9 show the relationship of the cord to other neighbouring structures. For convenience, all illustrations are shown as though taken from the right side.

- (1) The bicipital fossa of the ulna (*Bc.*; cross-hatched) is bounded by two ridges, both continuous inferiorly with the interosseous border (*I.*); superiorly, the anterior ridge passes to the side of the ulnar tuberosity and the posterior ridge approaches and may join the supinator crest (*S.C.*). The superficial head of the supinator arises in part from the supinator crest, whereas the deep head arises from the whole of the bicipital fossa.
- (2) In this specimen, there is no oblique cord, but the upper fibres of the deep head of the supinator form a distinct bundle (*B.*), which arises just lateral to the ulnar tuberosity; inferiorly, the bundle joins the remainder of the muscle.
- (3) In this specimen, the oblique cord (*Ch.*) is formed from a membranous sheet in contact with the upper part of the deep head of the supinator (*Su.*); fibres of the muscle arise from its deep aspect.
- (4) In this specimen, the anterior part of the deep head of the supinator is fibromuscular; the oblique cord is formed from the fibrous component and an additional fibrous strand is attached to the radius below the cord.
- (5) In this specimen, the anterior part of the deep head of the supinator is partly covered with membrane; the oblique cord lies in contact with its upper fibres.
- (6) The oblique cord is developed as a band in relation to a special bundle of the supinator; it is blended proximally with the flexor digitorum profundus (*Pf.*).
- (7) In addition to the oblique cord, there is a membranous band (*Ad.*) which gives way to muscle fibres inferiorly, and these are distributed between the flexor digitorum sublimis (*Sub.*) and flexor pollicis longus (*Pl.*). A fusiform 'occasional' head of flexor pollicis longus (*Occ.*) is also present.
- (8) The oblique cord is formed as a thickening of the fascia overlying the deep head of the supinator; it is in contact with the insertion of the supinator (*Su.I.*). An additional muscular head of flexor pollicis longus (*Ad.*) is present and it is blended proximally with the supinator and flexor digitorum profundus.
- (9) The oblique cord is in contact with the insertion of the supinator. An additional head of flexor digitorum sublimis is present, which is membranous and is blended proximally with the oblique cord.

Key to lettering of Figure 1

<i>Ad.</i>	Additional head of flexor pollicis longus or flexor digitorum sublimis.
<i>B.</i>	Separate bundle of deep head of supinator.
<i>Bc.</i>	Bicipital fossa of ulna.
<i>Bi.</i>	Tendon of biceps.
<i>Br.</i>	Brachialis.
<i>Ch.</i>	Oblique cord.
<i>I.</i>	Interosseous border of ulna.
<i>Occ.</i>	Occasional head of flexor pollicis longus.
<i>Pf.</i>	Flexor digitorum profundus.
<i>Pl.</i>	Flexor pollicis longus.
<i>S.C.</i>	Supinator crest.
<i>Su.</i>	Deep head of supinator.
<i>Su.I.</i>	Insertion of supinator.
<i>Sub.</i>	Flexor digitorum sublimis.

THE LIGAMENTOUS STRUCTURES IN THE CANALIS AND SINUS Tarsi

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INTRODUCTION

Between the talo-calcaneo-navicular joint in front and the talo-calcanean joint behind is an infundibular region which in the Birmingham Revision of the Basle *Nomina Anatomica* is termed the *sinus tarsi*. In a detailed consideration of the part it has been found advantageous to limit the term *sinus tarsi* to the wide lateral part of this region and to refer to the narrower medial part as the *canalis tarsi*. This is the terminology adopted in older works on the foot, and precedent for its re-adoption is to be found in the writings of Wood Jones (1944).

The form and the relations of the several ligamentous structures which lie in the canalis and sinus tarsi are to some extent controversial and the anatomy of these regions has consequently been examined in twenty-two feet. Of these, eight were fresh specimens from amputated limbs while the others were obtained from the dissecting room.

METHOD

Each foot was dissected by a modification of the method used by Barclay Smith (1896) in his investigation of this region. The foot and lower third of the leg were frozen and cut on a bandsaw. The plane of section (Text-fig. 1) was vertical and orientated so as to pass through the lateral and medial tubercles of the talus and thus behind the canalis and sinus tarsi. The posterior part of the specimen was discarded and the soft tissues of the anterior part of the leg above the inferior extensor retinaculum were removed, together with the fibula and the greater part of the tibia (Text-fig. 1). Thereafter the sinus and canalis tarsi were exposed by the piecemeal removal of those parts of the talus and the calcaneum which form their posterior wall, and the contents of the two regions were then demonstrated by sharp dissection.

OBSERVATIONS

In each specimen three ligamentous structures were found to lie within the sinus and canalis tarsi.

(1) *The inferior extensor retinaculum*

That part of the retinaculum which lies in front of the ankle has been fully described by Frazer (1920). Traced laterally the retinaculum turns downwards round the lateral aspect of the neck of the talus, to which it is sometimes attached (Pl. 1, figs. 2, 3), and enters the sinus tarsi. Within the sinus it divides into well-defined lateral, intermediate and medial roots (Pl. 1, fig. 1). The lateral root becomes incorporated in the deep fascia on the lateral aspect of the foot, the larger intermediate root descends vertically to the calcaneum and the slender medial root

inclines into the canalis tarsi before being attached to the sulcus calcanei. Between the intermediate and lateral roots is a triangular interval, occupied by fibres of the extensor digitorum brevis, whereas the intermediate and medial roots are separated by a semicircular gap which is bounded above by arcuate fibres.



Text-fig. 1. The posterior aspect of the anterior part of the leg and foot after sectioning. The black areas indicate the bone removed in the exposure of the structures in the canalis and sinus tarsi.

(2) *The cervical ligament*

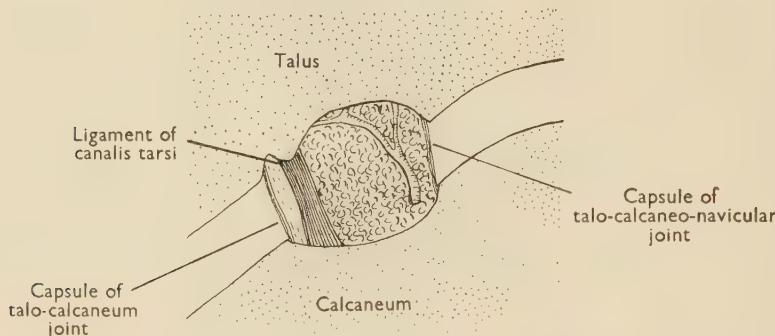
The second constant structure is a ligament lying in the anterior part of the sinus tarsi. A number of different names have been applied to it but the term *cervical ligament* which was used by Wood Jones (1944) is now widely accepted. In Pl. 1, fig. 4, the anterior segment of a right foot is seen from the supero-lateral aspect. The inferior retinaculum has been turned back after division of its medial attachments and the cervical ligament is seen as a broad, flattened band which lies anterior to the intermediate root of the retinaculum. It is attached below to the dorsal surface of the calcaneum medial to the extensor digitorum brevis and extends medially and upwards to a tubercle on the infero-lateral aspect of the neck of the talus.

(3) *The ligament of the canalis tarsi*

It was found that another ligament was constantly present in the canalis tarsi (*LCT* in Pl. 1, figs. 1-3 and 6-8). This ligament extends between the talus and the calcaneum, but it will be referred to here as *the ligament of the canalis tarsi* to avoid

the measure of ambiguity which is at present involved in the use of the term *interosseous ligament*.

The ligament of the canalis tarsi is a broad band, flattened in the coronal plane, in which the fibres extend downwards and laterally from the sulcus tali to the sulcus calcanei, crossing in their course either anterior or posterior to the medial root of the inferior extensor retinaculum. The site of its distal attachment shows no distinguishing features on the dried calcaneum, but its proximal attachment is to a sharp bony ridge which was a prominent feature in twenty-two of the thirty tali which have been examined. The ridge (Pl. 1, fig. 5) lies on the medial part of the sulcus tali, in front of the large vascular foramina which mark that area and immediately behind the sustentacular facet.



Text-fig. 2. Diagrammatic representation of a sagittal section through the canalis tarsi.

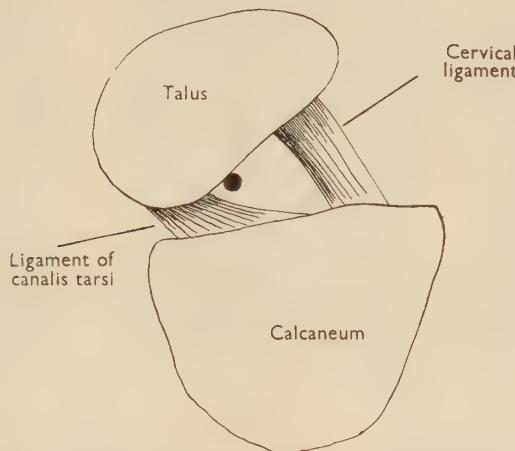
The ligament intervenes between, and is distinct from, the thin and partially deficient capsular ligaments of the talo-calcanean and talo-calcaneo-navicular joints: it is separated from the latter by a thin lamina of areolar tissue and from the former by a wider mass of highly vascular fatty tissue which lies below the large foramina on the sulcus tali (Text-fig. 2). This is very obvious, even in foetal specimens, and may be seen in Pl. 1, fig. 6, which is a sagittal section through the canalis tarsi of a 170 mm. c.r. foetus. This section passes through one of the deficiencies in the capsular ligament of the talo-calcanean joint.

In the majority of the specimens examined the ligament was quite separate from the cervical ligament, but in one, Pl. 1, fig. 7, it was joined to the latter by an oblique band, and in another, Pl. 1, fig. 8, the two ligaments were continuous.

FUNCTION OF THE LIGAMENTS

It is apparent that both the cervical ligament and the ligament of the canalis tarsi are mechanically associated with talo-calcanean movement. If, before any dissection is carried out, the compromise axis of this movement is determined by trial and error and the line of the axis is then marked by a pin driven through the head and neck of the talus, it is found after dissection is complete that the axis is related to the two ligaments as shown in Text-fig. 3. It follows from this relationship

that the cervical ligament is concerned with the limitation of inversion, whereas the function of the ligament of the canalis tarsi is to assist in the limitation of eversion.



Text-fig. 3. Diagrammatic representation of relationship of the axis of talo-calcanean movement (black dot) to the ligaments of the canalis and sinus tarsi.

DISCUSSION

The observations in this investigation conform very closely to the classical description given by Barclay Smith (1896). He described the structure here designated the ligament of the canalis tarsi as the oblique astragalo-calcanean ligament and recognized its variable relationship to the inferior extensor retinaculum. Despite this observation, however, he considered it to be part of the capsular ligament of the talo-calcaneo-navicular joint, rather than the distinct entity it is shown to be in Pl. 1, figs. 3 and 6. Furthermore, his statement that the ligament produced no visible markings on the bones to which it is attached is not in keeping with the present observations. On the other hand, the findings in this investigation show little similarity to the account given by Fick (1904): the only specimen which approaches his description is that shown in Pl. 1, fig. 8. More recently Wood Jones (1944) considered that the ligament of the canalis tarsi was simply part of the applied capsular ligaments of the talo-calcanean and talo-calcaneo-navicular joints. Last (1952) recognized the presence of a fairly strong and discrete ligament in the canalis tarsi, but maintained that it always consisted of two bands diverging upwards from the calcaneum to the talus. Such a description is not applicable to the majority of the specimens which have been examined. It might be applied to a specimen such as that shown in Pl. 1, fig. 3, in which the medial root of the inferior extensor retinaculum has a firm attachment to the neck of the talus, but comparison with other specimens suggests that such a description is unsatisfactory.

SUMMARY

1. The ligamentous structures in the canalis and sinus tarsi have been observed in twenty-two feet.
2. The sinus tarsi contains the cervical ligament and the lateral and intermediate roots of the inferior extensor retinaculum.
3. The canalis tarsi contains the medial root of the inferior extensor retinaculum and a structure which has been designated the ligament of the canalis tarsi. The canalis is limited anteriorly and posteriorly by the thin and partially deficient capsular ligaments of the neighbouring joints.

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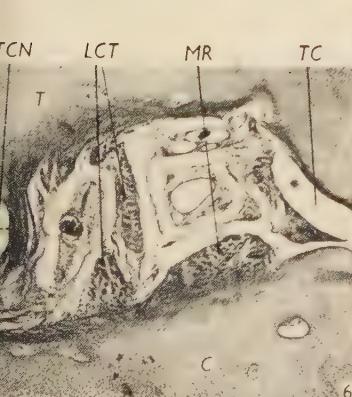
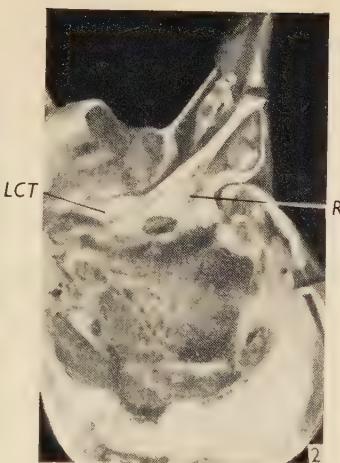
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EXPLANATION OF PLATE

Fig. 1. Posterior aspect of anterior segment of right foot.
 Fig. 2. Posterior aspect of anterior segment of right foot.
 Fig. 3. Posterior aspect of anterior segment of right foot.
 Fig. 4. Supero-lateral aspect of anterior segment of right foot.
 Fig. 5. Inferior aspect of left talus. The arrow indicates the bony ridge to which the ligament of the canalis tarsi is attached.
 Fig. 6. Sagittal section through canalis tarsi of 170 mm. human foetus.
 Fig. 7. Postero-lateral aspect of anterior segment of right foot.
 Fig. 8. Postero-lateral aspect of anterior segment of right foot.

Abbreviations

C, calcaneum; *T*, talus; *R*, inferior extensor retinaculum; *MR*, medial root of inf. ext. retinaculum; *IR*, intermediate root of inf. ext. retinaculum; *LR*, lateral root of inf. ext. retinaculum; *CL*, cervical ligament; *LCT*, ligament of the canalis tarsi; *TCN*, capsular ligament of the talo-calcaneo-navicular joint; *TC*, cavity of the talo-calcanean joint; *EDB*, extensor digitorum brevis.



THE SERIAL SECTIONING OF UNDECALCIFIED ANIMAL HEADS

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A number of techniques have been described for the sectioning of undecalcified bone (McLean & Bloom, 1940; Axelrod, 1947; Bertrand, 1948; Arnold, 1951; Arnold & Jee, 1954; Duthie, 1954; Woodruff & Norris, 1955), usually in preparation for radioautography. Here is described a method of serial sectioning of the undecalcified heads of young animals, preserving the bones *in situ* and in their normal relationship to one another.

The fresh heads are fixed in neutral 10% formalin and dehydrated through a series of neutralized ethyl alcohols (Axelrod, 1947). They are then infiltrated with and blocked in Ester wax as described by Steedman (1947) and Duthie (1954). About 3–4 weeks are required for infiltration of the largest specimens—some of which were sectioned in a parasagittal plane to facilitate penetration of the wax.

Sectioning is carried out on a heavy-base sledge microtome using a Jung extra-hard, tool-edged knife. ‘Sellotape’ is pressed on to the surface of the trimmed block before sectioning, and when the section is cut, it remains adherent to the tape. Several sections may be cut on to a single length of tape, at any desired thickness. At 10 μ there was a tendency to cut thick and thin sections alternately when there were areas of high calcification such as the tips of the incisor teeth. For the present purpose sections of 10, 50, 100 and 200 μ were found satisfactory. The sections can be stored indefinitely face down on covered trays.

Two methods have been used for mounting. In one, the sections on the tape are de-waxed by immersion in ethylene glycol monobutyl ether at 55°C. for 24 hr., then placed in isopropyl alcohol. From there, individual sections, still on the tape, are carried through a brief immersion in xylol and mounted immediately in Canada balsam, face downwards on the slide. No adhesive is necessary. More balsam is required between tape and cover-slip, and the latter should be wet with xylol. If held too long in xylol, the sections fragment on the ‘Sellotape’ as the adhesive is dissolved. Complete clearing proceeds on the slide. These sections were unstained except for intravital Alizarin Red, given at various periods prior to death by intraperitoneal injection (Schour, 1936).

In the other method, the sections on the ‘Sellotape’ are mounted on dry, albumen-coated slides on a hot-plate, pressed down with filter-paper and left for 15–20 min. The tape can then be floated off by 18–24 hr. immersion in xylol, de-waxing completed by two further changes of xylol (20 min.), and the sections treated in the usual way prior to staining, without need of celloidin coating. 50 μ sections were found to show good histological detail for routine examination. For comparison,

alternate sections can be decalcified on the slide by the use of 5% tetra-acetic acid before staining (Vincent, 1955). Using 'Sellotape', there is no significant difference in the size of the specimen in the block and on the slide.

SUMMARY

Whole young animal heads, blocked in ester wax, were serially sectioned with a hard knife by pressing 'Sellotape' on to the surface of the block. The sections adhere to the tape. They may be mounted without removing the tape, or, adhering to albumen-coated slides, the tape may be removed in xylol, and staining carried out in the usual way.

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JAMES COUPER BRASH

IN MEMORIAM

JAMES COUPER BRASH, M.C.; M.A., M.B., Ch.B. (Edinb.); M.D. (Birm.);
Hon.D.Sc. (Leeds); LL.D. (St Andrews); F.R.S.E.; F.R.C.S. (Edinb.)

Professor Emeritus James Couper Brash died at Edinburgh on 19 January 1958 in his 72nd year. The son of a bank manager, J. C. Brash was born at Helensburgh in 1886 and was educated at the Bell Baxter School, Cupar, while his family lived nearby at Leslie in Fife, and George Watson's College at Edinburgh.

In 1903, the year that D. J. Cunningham was appointed to the Chair of Anatomy at Edinburgh and the erstwhile professor, Sir William Turner, became Principal and Vice-Chancellor, Brash entered Edinburgh University and rapidly proved an outstanding student both in his academic work and in a wide range of extracurricular activities. Thus, though he graduated M.A. in 1906, B.Sc. with special distinction in Zoology in 1908, and M.B., Ch.B. in 1910 with distinction in each of the first three professional examinations, and was awarded the Sibbald Medical Scholarship and Baxter Scholarship in Natural Sciences, yet he was able to be Secretary and Senior President both of the Philomathic Society and the Students Representative Council, Secretary of the University Physiological Society, an active member of the Committee of Management of the University Union, a student demonstrator both in Anatomy and Pathology and Treasurer and Vice-President of the Liberal Association. In the latter capacity he took a considerable part in the Rectorial Election of 1908 when the Liberal candidate, Winston Churchill, and Sir William Osler were narrowly defeated.

Following a year in the medical wards of the Royal Infirmary, Edinburgh, he was appointed a Demonstrator in Anatomy by Prof. Arthur Robinson, but proceeded almost at once to the Anatomy Department in Leeds where he served under Prof. J. K. Jamieson till his mobilization as a member of the Special Reserve in August 1914, by which time he was already recognized as a skilful anatomist and an excellent lecturer.

Throughout the First World War he was on active service with the R.A.M.C. in France and Belgium, and although he was attached to the 10th Field Ambulance for the greater period of his service, he was also M.O. to the 1st Royal Warwicks and the 2nd East Lancs. In 1916 he was awarded the M.C. for services in the field and was later promoted to the rank of major.

Demobilized in 1919, he returned to Leeds and within six months was appointed Assistant Professor of Anatomy at Birmingham where he was soon to take over most of the work of the Department owing to the illness of Prof. Peter Thompson. This situation was recognized in 1921 when he was appointed Acting Professor and, in the following year, after the death of Prof. Thompson in November 1921, he was appointed to the Chair at Birmingham University where he remained for a further nine years. In Birmingham he was Dean of the Faculty of Medicine from 1928 to 1931 and it was in this capacity that he was responsible for the planning of many of

the developments that led to the formation of the new Medical School there. For such a task he was pre-eminently qualified and his width of vision and his meticulous attention to detail are expressed in much of the stone and mortar that form the fabric of this Medical School today. In 1931 he returned to Edinburgh in succession to Arthur Robinson in the Chair of Anatomy, and remained there till his resignation in 1954.

During his period in Birmingham he was extremely active both in teaching and research and spent much of his time in the dissecting room. He published a long series of papers on a wide range of subjects, including anthropological studies on material obtained from Anglo-Saxon burials, and in conjunction with Prof. Thompson he described an early human embryo. It is noteworthy that the first paper he published in this post-war period was in collaboration with the late M. J. Stewart, Professor of Pathology at Birmingham, who was to remain a lifelong friend. Yet through all this he was developing his main interest, the growth of bone and particularly the normal and abnormal development of the jaws and the movements of the teeth; studies which he continued up to and after his retirement, and which were to make him an authority on the subject and win him world-wide recognition among orthodontists. Thus he was elected to honorary membership of the British Society for the Study of Orthodontics, the British Dental Association, the European Orthodontic Society and to Honorary Fellowship of the Odontological Section of the Royal Society of Medicine. Such was his reputation that on more than one occasion children with orthodontic problems were brought to see him from the United States. It was characteristic of him that he was vastly intrigued by this and always assured the parents that he had no special knowledge of the subject.

In a series of publications between the years 1924 and 1929, he dealt with many aspects of the growth of the jaws and the movements of the teeth, and in 1929 gave four lectures for the Dental Board of the United Kingdom on the 'Aetiology of Irregularity and Malocclusion of the Teeth'; these were later (1931) published in book form and demonstrated the wide range of his knowledge, his clarity of expression and above all the scientific approach to a subject in which too often empiricism had held sway. It is a tribute to the value of these lectures and their enduring importance that soon after his retirement Brash was called upon to revise them for publication of the second edition which appeared in 1956. Throughout the intervening years he continued his work on this subject, publishing in the same year 'A Preliminary Note on the Mode of Growth of the Mandible' and following this with the Sixth Sir John Struthers Lecture at the Royal College of Surgeons of Edinburgh entitled 'Some Problems in the Growth and Developmental Mechanics of Bone'. The last of his studies on bone growth was concerned with the processes involved in the movements of the teeth in the jaws, and in particular with the remarkable arrangements in the dugong, manatee and elephant which he was able to elucidate by a close investigation of the appearances of the alveolar bone, demonstrating the precise sites of deposition and re-adsorption of bone which are responsible for the continuous movement of the teeth within the jaws. The results of these many studies he brought together in the Sixth Northcroft Memorial Lecture entitled 'The Comparative Anatomy of Tooth-Movement during Growth of the Jaws' (1953). His interest in these developmental problems led him to prepare and study an extensive series of madder stained bones and to enter into prolonged experiments with

various anthraquinone dyes which it was hoped to use in the treatment of osteomyelitis. Indeed many of these were used on patients, and though some of the results were promising this particular approach to the treatment of bone infections was doomed to proceed no farther on account of the development of other chemotherapeutic agents.

During the Second World War, the need for detailed information regarding the exact points of entry of vessels and nerves into muscles in connexion with the many peripheral nerve injuries of the limbs, led him to study, by dissection, the neuro-vascular hila of the muscles of the 'extremities'. The results obtained on the upper limb were produced in the form of an illustrated brochure and circulated privately to peripheral nerve injury centres, but it was not until 1955 that the completed work was published in book form as an atlas of the neuro-vascular hila of the limb muscles. This publication was the result of very many dissections which he carried out with the same care that he lavished on everything he did despite continued recurrences of an old eye trouble, which was not improved by exposure to formalin, and the increased teaching and administrative load which the war and the post-war developments brought.

In 1937, after a long series of investigations concerned with the identification of the remains which were found near Moffat and sent to the Anatomy Department at Edinburgh, he published, with Prof. J. Glaister, *The Medico-Legal Aspects of the Ruxton Case*, for which he received the Swiney Prize of the Royal Society of Arts. While he was well aware of the limitations of the evidence which he produced, he was responsible for the development of the method of matching the radiographs of the skull of Mrs Ruxton to a photograph which had been enlarged to life size. This same technique he later applied to the busts of William Burke, the notorious murderer and thus identified the genuine from the spurious casts which were produced at the time of Burke's execution for sale to the general public. At the time of his death Brash was concerned with a similar problem, namely that of attempting to reconstruct the head of King Robert the Bruce with a view to the production of an accurate likeness for a statue which it is proposed to erect on the field of the Battle of Bannockburn. To this end he had approached a well-known Edinburgh sculptor, who was already interested in the project, with the suggestion that he might make use of the cast of the reputed skull of King Robert the Bruce and build on to it features commensurate with the skull. His interest in these matters was no doubt conditioned in part by the fact that for 24 years he was Chairman of the William Ramsay Henderson Trust, a Trust founded and endowed by Ramsay of Eldon and Warriston for the study of phrenology and which was actively concerned with neurological matters during the period of his Chairmanship. He was editor of two of the Trust's publications, *The Hypothalamus* by Le Gros Clark, Beattie, Riddoch and Dott, in 1938, and *Prefrontal Leucotomy and Related Operations* by Meyer and Beck, in 1954. Had it not been for the intervention of the war there would have been many similar publications edited by him.

Brash had all the characteristics of a first-class editor, and the time, effort and care which he bestowed in revising no less than three editions of *Cunningham's Text-book of Anatomy* and four editions of the *Manuals of Practical Anatomy* were in no small measure responsible for the continued popularity of these publications.

All this he carried on in addition to serving on a great number of committees, not only in the University but also in the Royal Infirmary of Edinburgh and elsewhere, and to all of these he brought the same energy and meticulous attention to detail combined with a remarkable ability to get to grips with the essentials of each and every problem with which he was faced.

Brash was a man of wide interests and with a profound knowledge of the history of his subject and especially of the Edinburgh Medical School. It was this that prompted him to travel round the world only one year after his resignation from the Chair at Edinburgh, with the particular purpose of seeing at first-hand the Monro documents which are housed in the University of Dunedin Library. Despite a serious illness after his return to this country he set about the arduous task of collecting and annotating all the Monro documents with a view to their publication by the University of Edinburgh Press. It is a great misfortune that he was unable to complete this work on the founders of the Edinburgh Medical School, on which he was engaged right to the end, for there was no one better qualified than Brash to carry out this difficult task.

The list of his publications is long and, though it contains many reviews and obituary notices, those who knew him realized the immense amount of work that he put into each of these tasks, for in all that he did he showed the same vigour, the same close attention to detail and an overriding passion for accuracy which was his outstanding characteristic.

It is difficult to paint a picture of Brash the man, for he was unobtrusive in the things that he did and went out of his way to hide the many kindly acts which he carried out for all those in contact with him. His thoughtfulness for others is exemplified by the fact that he prepared the bibliography attached to this notice and left it among his papers expressly to save the writers the difficult task of gathering this information together.

On the Edinburgh Medical School Professor Brash left a deep and lasting mark. His experience as an administrator was of infinite value to the Deans of both Medicine and Science and his advice on many administrative problems was frequently sought. His professional and other colleagues at Edinburgh will long remember him as a man of outstanding character and the close and friendly relationship that he established with many of them was characteristic.

A keen and expert fisherman and a golfer to be reckoned with, a man of great personal charm and a delightful companion full of pawky humour and a fund of information on a diversity of subjects, a wise counsellor and a kindly examiner, Brash had a host of friends and interests outside the University. Many anatomists will remember him with pleasure as a most amiable host not only to the Society as a whole when, in 1947, he was President but also at his home where he was so ably assisted by his charming wife. To Mrs Brash and to his son and daughter we extend the most sincere sympathy in their recent loss.

Brash died as he lived, with the best interests of Anatomy always in the forefront of his mind, for he bequeathed his body for the purposes of dissection and left instructions that his Bequest should be adequately publicized, so as to stimulate others to follow his example. It would indeed have pleased him to know the response which his final act evoked.

G.J.R.

R.W.

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REVIEWS

A Student's Histology. By H. S. D. GARVEN, B.Sc., M.D., F.R.S.E., F.R.F.P.S. (G.). (Pp. xii + 650; illustrated; 55s.) Edinburgh and London: E. and S. Living-stone. 1957.

A new 'Histology' on the British market is certainly an event and to be welcomed as such. It doubtless is the expression not only of a need, but also of the renewed importance which this subject has been receiving in recent years. Long neglected and relegated almost to the role of a Cinderella with tattered rags and fading colours she has, clad in the glittering garments provided by the good fairies Histochemistry and Electron Microscopy, suddenly made her appearance in the highest societies and is wooed by many of their members, though not declared a queen in her own right. However, it is not with the glittering garments that Dr Garven concerns himself, but rather with the solid foundations of straightforward light microscopy which is, after all, essential and indispensable for a students' text. Yet some selected references to Histochemistry which is helping to narrow the gap between structure and function, and especially to Electron Microscopy which has clarified some important controversial morphological issues of long standing, would not have been altogether out of place.

When the author says in his foreword 'to the student' that he 'is more concerned to give ... a point of view than a detailed compendium of facts', he does himself an injustice. For the amount of macro- and micro-anatomical details and of physiological data amassed in this volume is quite considerable, and there is much the student can read with great benefit. The first chapter on The Cell and General Considerations is very good; the note, in small print, opening the chapter on haemocytopoietic and lymphatic organs is a gem, and the R.E.S. is dealt with better than in any other text-book; other chapters which read well are those concerned with the small and large gut and the endocrine glands, where a good balance is kept between morphology and function. Emphasis is rightly put on vascular supply, lymphatics and organ innervation. As many as 16 pages are devoted to the innervation of the skin; and later we are made to follow the morphological pathways of a somatic and an autonomic reflex arc, stopping *en route* in nerve trunks, ganglia and at other places, though no description is given of a representative section of the spinal cord, let alone cerebellum and cerebrum.

However, omissions, on the whole, are few; it is more regrettable that the presentation is not always clear and accurate, but this can be remedied in a future edition. It would pay the author to comb his text, not only to eliminate a number of rather confusing printing errors and inaccuracies, but also, by following the logical and grammatical link from sentence to sentence, to clarify the text.

Often a paragraph headed 'general', occasionally followed by another headed 'general structure', precedes the more detailed description of an organ, but already contains a nomenclature which presupposes knowledge of the intimate structure. The passages dealing with embryology are usually too brief and superficial to be helpful and illuminating, and complicated developmental processes are described without illustrations. It is strange to see the epithet 'polyhedral' applied to fibroblasts; a 'large eccentric' nucleus allotted to the plasma cell; to read of dentine as a highly sensitive cell-free matrix; of 'true' nerve cells; and of the medullated nerve fibres in the C.N.S. as being covered by neurilemma.

The chapters on epithelia and supporting tissues leave much to be desired. The classification of epithelia loses its value by an enumeration of almost every conceivable type; simple and compound epithelia get mixed up, and endothelium is thrown in for good measure. This confusion later pervades the text at various places and is particularly evident in the account of the eye and the inner ear. The presentation of a general plan of the digestive tube prior to dealing with the mouth cavity, where almost nothing of this general

pattern holds, is a feature which this book has in common with most others on the subject. Similarly, no submucosa is conceded to ureter and bladder; only part of the cornea is continuous with the sclera, and the conjunctiva is more than merely its epithelium.

As for illustrations, the author decided to use almost exclusively diagrams. Many of these are good as such, some are even exquisite, but the majority is so far removed from reality as to be of little help to the student when examining actual specimens. Some bear no relation to histology at all. There are a few nice coloured plates which illustrate the outcome of different staining methods. For the islets of Langerhans Gomori's stain would have been preferable to Mallory's. May I point out two oversights in annotation? In Fig. 8, 10 what is enamel pulp is labelled 'loose connective tissue'; and in Fig. 12, 22 'endoneurial sheath' should be replaced by 'neurilemma'.

At a time when classical education is sadly neglected or frowned upon, it is pleasing to see the Greek and Latin derivation of technical terms; but why only of a privileged few?

There are two appendices, the one giving a fair selection of references, the other listing famous histologists whose names and contributions should rightly be kept alive.

F. JACOBY

Biochemical Cytology. By JEAN BRACHET. (Pp. xi + 516; 190 illustrations; \$8.80.)
New York: Academic Press.

This book attempts, in the author's own words, '...to present an integrated version of the facts which to-day are known about the morphology and the biochemistry of the cell'. In many ways the attempt is successful. Data from many different experimental disciplines are collected together and their bearing on the major problems of cell cytology critically discussed. For some aspects of this subject—particularly those concerning the nucleic acids and protein synthesis—the author here provides probably the best up-to-date account available in book form. A major criticism that might be made and one which the author freely admits in his preface is that the material balance of the book is very much a reflexion of his own interests and experience. It is, however, just this frequent preoccupation of the author with his own special interests that makes this book so readable and prevents it ever becoming a mere catalogue of facts.

The book is divided into ten chapters of very unequal length. The first two are short and give the reader a brief, historical sketch of the subject and a summary of the types of techniques available. The following six chapters form the bulk of the book, totalling as they do some 400 pages. The first two of these chapters provide a very adequate account of the cytology of the resting cell from both the morphological and the biochemical viewpoints. The next chapter deals with mitosis, and here the emphasis is largely a biochemical one. Chapter six is almost entirely devoted to a detailed discussion of the evidence, drawn from many sources, for the idea that RNA is intimately concerned in protein synthesis. It is, in many respects, the focal point of the whole book, and it includes an account of much recent work from the author's own laboratory. The remainder of this main section of the book deals with two special problems concerning particular types of cells: nucleo-cytoplasmic interactions in unicellular organisms; and the relative roles of nucleus and cytoplasm in embryonic differentiation. The final two chapters, a short discussion of cancer cells and a few concluding remarks, lack some of the force and inspiration of the rest of the book.

Each chapter ends with a list, usually a very long one, of the references cited. In view of the obvious value of this book as a guide to the cytochemical literature, it is unfortunate that there is no Author index; the Subject index, however, appears to be very complete. The book is excellently produced and well illustrated, the quality of paper being such that it has been possible to reproduce photomicrographs in the body of the text where they are most relevant.

That the author has chosen to write this book directly in English is both a compliment and a challenge to British and American scientists; but it is, perhaps even more so, a sad

reflexion of our failure to read even important books which happen to be published in a foreign language. Certainly, Prof. Brachet's command of the English language is quite adequate for the task he set it: only occasionally are words misused, and seldom in such a way as to interrupt the reader's train of thought. Much of the book presupposes an acquaintance with cytology, biochemistry and related subjects; so, for the undergraduate student of biology, it will probably be most useful in his final year. It should prove particularly valuable to the postgraduate student of cytology and, in fact, to anyone in teaching or in research who is concerned with living cells.

P. R. LEWIS

Sir Charles Bell, his Life and Times. By Sir GORDON GORDON-TAYLOR and E. W. WALLS. (Pp. xii+288 and 49 figures. 42s.). Edinburgh and London: E. and S. Livingstone Ltd.

The title of this book is fully justified by its contents. In addition to giving a full account of the life of Charles Bell, the authors have contrived to etch in with well-chosen, firm but delicate strokes the background against which that life was passed. A chapter depicting the London of Charles Bell and others on his eminent contemporaries and on the surgical practice of his time serve to illuminate aspects of Bell's career which might otherwise slip the attention of the general reader. Approximately one-third of the total text is occupied by a valuable series of appendices, perhaps the most interesting of which is formed by the collection of letters relating to cases seen by Bell after the Battle of Waterloo. These revealing documents provide an admirable foil to the beautiful reproductions of Bell's own water colours which are amongst the many excellent illustrations gracing the pages of this elegant volume.

At first sight the life of Charles Bell seems to possess all the ingredients familiar to readers of the biographies of eminent Victorian medical men. The precursors of triumph are certainly there; the good Lowland stock, the adored and adoring widowed mother, the impoverished childhood, the precocious exhibition of talent and the inevitable early setback (in Bell's case his failure to obtain the Professorship of Anatomy at the Royal Academy when the outstanding candidate). Yet his professional life as it unfolded itself showed sombre overtones more characteristic of the age of revolution and doubt in which it was largely spent than of the era of affluent optimism in which it was to close. His success when it came was curiously incomplete. If he found fame, he failed to win fortune, and his widow was provided for by a Civil List pension. To some extent, as his biographers point out, his failure to gain affluence was an inevitable result of his choice of specialty and preoccupation with research. His somewhat unfortunate decision to return to Edinburgh in the autumn of his career rounded off a life in which the strands of joy and woe were always finely interwoven.

Roux's dismissal of his class with the famous sentence, 'C'est assez, messieurs, vous avez vu Charles Bell', illustrates the reverence in which Bell was held by his contemporaries, particularly on the Continent. Sir Gordon Gordon-Taylor and Professor Walls clearly share this affection for their subject to the full, but they have not allowed it to blind them to his defects. The most serious of these, his lack of generosity to Mayo, they attribute to the jealousy of the financially unsuccessful, and in the controversy with Magendie they follow Olmsted in awarding the palm to the Frenchman. If one cannot but admire their restraint and concur with their judgement, one does so with reluctance, for Charles Bell was, above all else, a man of whom one might truly say *bonum facile crederes, magnum libenter.*

D. H. M. WOOLLAM

PROCEEDINGS OF THE ANATOMICAL SOCIETY
SEPTEMBER 1957

An additional ordinary meeting of the Society for the Session 1956-7 was held on Friday, 27 September 1957, at the Royal Society of Medicine, Wimpole Street, London, W.I. The President (Prof. R. D. LOCKHART) was in the Chair.

The following are the authors' abstracts of the papers read.

A study in quantitative cytoarchitectonics. By D. A. SHOLL.
University College, London

The results of attempts to divide the cerebral cortex into areas of differing anatomical organization are inconsistent mainly because they are based on subjective criteria and fail to consider the variation that is present between the cortices of adult brains of the same species. The present study overcomes these difficulties by using the concept of Generalized Distance due to Mahalanobis (*Proc. nat. Inst. Sci. India*, 2, 1936). This is a measure of the degree of resemblance between groups of observations subject to variation, and has all the properties of Euclidean distance.

The investigation has been made on Nissl-stained preparations of various regions of human, cat and mouse cortices. The number of neurons per unit volume of cortex is estimated at each of ten different relative depths; the absolute thickness is an eleventh variable. The counts have been made on sections from different adult brains of the same species and also replicated on sections from the same brain. The Generalized Distances between the different cortices have been computed from a consideration of the variances and correlation coefficients between the observed values of the different variables.

The results show, for example, that the cortices from each species tend to form a cluster; that the human parastriate cortex is 'equidistant' from human motor cortex and from human visual cortex and that mouse visual cortex is almost as 'distant' from human visual cortex as it is from human motor cortex. It is possible to attempt to relate these and similar results to possible 'functional specialization' in the different cortices.

No account has been taken of differences in neuron type of connectivity; these additional variables will be considered in a further study.

Some effects of temperature upon the rate and progress of Wallerian degeneration in mammalian nerve fibres. By H. J. GAMBLE and B. D. JHA, *St Mary's Hospital Medical School, London*

Diverse views have been held concerning the point of onset and the subsequent progress of the degenerative process in injured nerve fibres. The two most recent investigations have led to opposite conclusions. Erlanger & Schoepfle (*Amer. J. Physiol.* **147**, 1946) have claimed that breakdown in conduction is random, with increasing frequency peripheralwards or with failure proceeding centripetally, while Causey & Palmer (*J. Anat., Lond.*, **87**, 1953) have claimed that an early change in the myelin sheath (the retraction of the myelin at the nodes) spreads centrifugally.

It is, however, well established that lowered temperatures, even in mammals, retard the degenerative process as a whole. Experiments have now been made in rats to alter the environmental temperature of a part of an injured nerve fibre, so that in some specimens the proximal part is warm and the distal part cold, while in others the proximal part is cold and the distal part warm.

Using temperature differences of the order of 15-20° C., and studying the injured fibres in teased, osmium tetroxide preparations, it has been shown that cold retards degeneration

whether applied proximally or distally, and that warmth accelerates the process, again whether applied proximally or distally.

These results are discussed in relation to those of other workers and reference made to the considerable temperature differences which have been recorded in the different parts of the body in mammals.

Arachnoid granulations in Man By E. R. A. COOPER. *University of Manchester*

This research began with the study of arachnoid villi and arachnoid granulations in the cat. It has been continued in the human subject. Serial coronal sections of the entire saggital sinus and relevant parts of the meninges and cerebral hemispheres have been prepared from the brains of adults, infants and monkeys. Foetal heads and cat heads have also been sectioned with meninges *in situ*. X-ray examination of the cranial and spinal subarachnoid space has been carried out in cats and monkeys. From these investigations it has been observed that the arachnoid villi and granulations are organized and orientated constantly at specific sites relative to the cortex and the sagittal sinus. Anatomical and histological details are given and suggestions are submitted as to the possible purpose of the arachnoid granulations in the light of these findings.

Technique for the study of microcirculation By P. A. G. MONRO.
University of Cambridge

The circulation in small blood vessels may be studied in specially prepared chambers inserted into appropriate parts of an animal such as the rabbit's ear or the dorsal skin of the mouse. In these transparent chambers the tissue is usually of the order $50-60\mu$ thick, though thinner ones will allow of the growth of capillaries only down to about 20μ .

The rate of circulation of blood cells may be seen easily by direct observation and has been recorded on to ciné film. A new method utilizing the surface of a specially bright cathode-ray tube as a source of illumination for microscopy enables the circulation to be seen by flashes of variable rate, duration and intensity.

There is a critical rate of flashing at which blood cells moving at a certain velocity appear to have no general direction but spatter in random fashion like raindrops on a window pane. Flashing at a slower rate gives the impression that the circulation is reversed: at a faster rate the circulation is in its correct direction. This technique provides a convenient method for estimating the relative velocity of blood cells in different areas. A demonstration is given of the technique as seen when applied to the rabbit's ear chamber. It can easily be adapted to naturally transparent parts of animals such as the bat's wing, hamster's cheek pouch or tadpole's tail.

Further observations on the 'carrying angle'. By F. L. D. STEEL.
London Hospital Medical College

In a previous communication the upper limb of the carrying angle was defined as the tangent to the head of the humerus passing through the tip of the medial epicondyle. In order to establish the significance of variations due only to the size of the epicondyle this line has been related to the long axis of the humerus in man and other anthropoid primates. Further measurements of the angle have been carried out on disarticulated human bones and those of apes.

The coronary arteries of the Bantu heart. By R. SINGER.
University of Cape Town, South Africa

Death due to coronary thrombosis among the Bantu-speaking South African negroids is rare. From a previous small series (Brink, A. J. *Clin. Proc.* 8, 1949) it was suggested that this may be accounted for by a distinctive pattern of distribution of the coronary

arteries of the Bantu heart. However, a survey of hearts from 83 Bantu, 109 Cape Coloured and 86 White South Africans indicates clearly that the so-called 'Bantu pattern' is an individual variation noted in all three racial groups. It is also pointed out that the present description of the branches of the coronary arteries is inadequate and that constant variants supplying the various areas of the myocardium should be further investigated. Furthermore, the confusing nomenclature of various authors should be revised and standardized.

Effects of dilute copper sulphate on the tyrosinase reaction in melanocytes of freckled human epidermis. By A. S. BREATHNACH. *St Mary's Hospital Medical School, London*

When a pure epidermal sheet containing a freckle and an adjacent paler area is incubated with a buffered 0·005M solution of L-tyrosine at a pH of 6·8, tyrosine activity is found to develop more rapidly and to a more intense degree (at all intervals up to 24 hr. incubation at 38° C.) in the melanocytes of the freckle as compared with those of the paler epidermis.

The presence of dilute copper sulphate in the substrate solution (0·4 ml. of 1% CuSO₄ per 50 ml.) leads to an initial speeding up of the reaction in the melanocytes of both areas, but would appear to have only a slight enhancing effect on the final intensity of the reaction. It does not abolish the above observed local differences in tyrosinase activity between the melanocytes of freckle and paler epidermis which suggests that these differences are not entirely due to a difference in the availability of copper in the two areas. The significance of these findings in relation to the 'Sulphydryl-inhibition' theory of the control of the melanogenic response of ultra-violet irradiation of human skin is discussed.

The distribution of ³⁵S in the hypothalamo-neurohypophysial 'neurosecretory' system of the rat following the intracisternal injection of ³⁵S-labelled DL-cysteine. By D. G. ARNOTT and J. C. SLOPER. *The London Hospital and Charing Cross Hospital Medical School, London*

On histochemical grounds it has been suggested that throughout the supraopticohypophysial tract there is a protein rich in cystine or cysteine (Sloper, 1954), for this reason possibly akin to posterior pituitary principle. The substance thus demonstrated is in the exact distribution of a 'neurosecretory' material (Bargmann, 1949; Bargmann & Scharrer, 1951) supposedly synthesized in the supraoptic and paraventricular nuclear regions, and passed down the tractus hypophyseus to be stored in the infundibular process of the posterior lobe of the pituitary. With a view to studying the dynamics of this 'neurosecretory' system, the distribution of ³⁵S has been investigated in the hypothalamus and pituitary of the young rat at various intervals after the injection of ³⁵S-labelled DL-cysteine prepared from ³⁵S-labelled DL-cysteine obtained at Harwell. It was possible that this would be incorporated in 'neurosecretory' material, and concentrated in the hypothalamo-neurohypophysial system under investigation. Labelled cysteine was therefore injected both intraperitoneally and intracisternally, the second method proving the more satisfactory. Autoradiographs revealed a greater uptake of ³⁵S in the supraoptic and paraventricular nuclear regions than in the adjacent hypothalamus. This occurred within 30 min of injection. Maximal uptake by the infundibular process of the pituitary was noted at between 14 and 17 hr. The late uptake of ³⁵S by the posterior pituitary has been corroborated by quantitative measurements made with a gas-flow beta counter. These observations are consistent with the theory of the hypothalamic origin of 'neurosecretory' material in the hypothalamo-neurohypophysial system. It is stressed, nevertheless, that we do not know whether the radio-active sulphur taken up by this system is incorporated in protein: nor can it be assumed that this sulphur reaches the posterior lobe of the pituitary via its stalk.

Observations on the histochemistry and electron microscopy of the notochord.
By C. R. LEESON and T. S. LEESON. *University College, Cardiff*

Rabbit embryos of 13, 15 and 17 days have been investigated. At 13 days the notochord is present in a primitive, cylindrical form. The cells show early vacuolation and, although cell boundaries are difficult to define on light microscopy, they are clearly seen with the electron microscope. The sheath is well defined and appears to consist of collagenous or precollagenous fibres in a matrix containing acid mucopolysaccharides. Evidence of early segmentation, i.e. intervertebral dilatation and intra-vertebral constriction, is seen by the 15-day stage. The cells in the intervertebral regions are increased in number and no longer form a solid rod, spaces now being present between the cells. Segmentation is much more obvious by the 17-day stage, continuity of cells being lost in the developing centra, although the sheath remains intact. In the intervertebral regions, the cells form cords and strands arranged in a network which is referred to as the 'chorda reticulum'. Cell boundaries are still seen on electron microscopy and there is no evidence of a syncytial formation such as was described by Williams (1908) and Dawes (1930). In all stages, the sheath contains microfibrils which are more dense in relation to the perichordal tube. No outer limiting membrane to the sheath is seen in any of the stages examined.

Knife edges suitable for ultra-microtomy of hard tissues.By R. W. FEARNSHEAD. *London Hospital Dental School*

To cut ultra-thin sections of hard tissues for electron microscopy without preliminary softening presents special difficulties. The use of an industrial diamond fashioned into a knife edge was first described by Fernández-Morán (1953) who suggested the possibility of using such knives to cut hard materials.

Experimental diamond knives prepared by Dr Custers and the late Dr Paul Grodzinski have been used in this laboratory to cut calcified dental tissue for four years.

These knives differ very slightly in design from those prepared in Fernández-Morán's laboratory. Some observations resulting from experience gained in the use of both types of knives on hard materials are described. Because of the great difficulty in obtaining suitably prepared diamond knife edges, the maintenance of which requires special skill possessed by only a few expert diamond artisans, preliminary experiments in collaboration with E.M.I. (Hayes) have been made in the preparation of knife edges from artificial sapphires. These knives have the advantage that they may be prepared easily, at low cost and periodically resharpened by methods available in any laboratory.

An attempt to assess the quality of the various knife edges has been made using a surface-interference microscope kindly loaned for the purpose by C. Baker (Holborn). The quality of the sections obtained was examined by optical and electron optical methods. Vickers micro-hardness tests were made on some of the cut surfaces of the blocks in order to give some data on the comparative hardness of the tissues used for this study.

Observations on certain histochemical reactions of the foetal vaginal epithelium.
By D. BULMER. *University of Aberdeen*

In the hypertrophic vaginal epithelium of two older human foetuses of 180 and 375 mm. crown-rump length respectively, the intercellular material of the intermediate and superficial layers gives a positive PAS reaction which is resistant to previous salivary digestion. This is, apparently, similar to the reaction described in the adult vaginal epithelium by Wislocki, Fawcett & Dempsey (*Anat. Rec.* **110**, 1951).

In addition, the intercellular material in the foetuses under investigation is sudanophilic, even in formalin-fixed paraffin sections, and gives positive reactions with the peracetic and performic acid-Schiff techniques. After exposure to atmospheric oxidation there is a direct reaction with Schiff's reagent, and this oxidation is very much accelerated by the presence of ultraviolet light.

The PAS reaction is completely blocked by previous acetylation. The PAAS and PFAS reactions and the autoxidation-Schiff reaction are all unaffected by acetylation and reduced, though not abolished, by bromination. All the reactions with Schiff's reagent are completely abolished by a dimedone blockade, indicating the aldehydic nature of the oxidation products.

The significance of the results is discussed in relation to the constitution of the inter-cellular material and to the mechanisms of the histochemical reactions involved.

'Recruitment' as a growth factor in the human tooth germ.

By A. R. TEN CATE. *London Hospital Medical College*

The early dental literature describing the histology of the internal enamel epithelium reports cells of the stratum intermedium intermingling with cells of the internal enamel epithelium. No significance has been attached to this finding.

A study of the two cell layers in the human developing tooth has shown that cells of the stratum intermedium are capable of insinuating themselves between the cells of the internal enamel epithelium (prior to their becoming functional). Cells of the stratum intermedium approach the internal enamel epithelium and come to occupy an intermediate position between the two layers. Their nuclei exhibit a characteristic 'pear drop' morphology. At this stage, the intermingling cells lose their alkaline phosphatase activity. A pseudopodic process is emitted which extends between the cell interfaces of two adjoining internal enamel epithelium cells until the dentino-enamel junction is reached. The nucleus of the intermingling cell then follows the course of the protoplasmic flow until it attains the same level as the internal enamel epithelium nuclei. During the intermingling process, a prominent connection with the stratum intermedium is retained. There is evidence to suggest, but not to prove, that the described intermingling cells eventually assume an ameloblastic role. The functional significance of this finding is discussed and it is suggested that the phenomenon of intermingling represents a contributory factor to growth of the tooth germ.

The cells of Ranvier's ossification groove in the long bones of rat and human embryos. By C. W. M. PRATT. *University of Cambridge*

There are conflicting views concerning the origin of the cells found in Ranvier's ossification groove. Some workers have described these cells as arising from the chondroblasts of the epiphysis, while others have believed that the cells of the groove are concerned in chondrogenesis rather than being the result of chondrolysis. This disagreement results from the lack of knowledge of the precise arrangement of these cells and the changes that occur in the groove during development.

The present observations are based on the long bones of both rat and human embryos where the epiphyses appear to grow by the apposition of prechondroblasts which arise by the differentiation of adjacent mesenchyme. The epiphyseal border of the groove shows a variable amount of appositional growth; where the growth is marked the prechondroblasts are derived from the periosteal fibroblasts and the groove has a laminated arrangement of cells. In situations where little appositional growth is occurring at the epiphyseal lip of the groove, the groove contains an irregular arrangement of undifferentiated mesenchymal cells. Osteoblasts are found at the diaphysial border of the groove, they are derived from adjacent periosteal fibroblasts and are not affected by the type of cellular arrangement found in the groove.

NOVEMBER 1957

The annual General Meeting of the Society for the Session 1957-8 was held on Friday, 29 November 1957, in the Department of Anatomy, St Bartholomew's Hospital Medical College, London, E.C. 1. The Chair was occupied by the retiring President (Prof. R. D. LOCKHART) for the morning session and by the newly elected President (Prof. F. GOLDBY) for the afternoon session.

The following are the authors' abstracts of the papers presented.

Functional atrophy of maxillary origin. By A. J. E. CAVE.
St Bartholomew's Hospital Medical College, London

At harmonic sutures involving the maxilla the superjacent non-maxillary cranial element may undergo a natural and partial pressure atrophy, resulting in the appearance of a suturally-circumscribed exposed portion of the subjacent maxilla and falsely suggesting the presence of an adventitious cranial element. Examples are provided by elephant and rhinoceros crania.

In fully mature Asian and African elephants, the superficially disposed premaxilla may be so 'eroded' as to manifest an apparent 'ossicle' with sutural boundary, which is merely part of the underlying maxilla. The causative factor of such localized premaxillary pressure atrophy is the progressive expansion of the maxillary sinus. This, at still later age-stages, may even fenestrate the surface of the exposed portion of the maxilla.

In all five extant rhinoceros species, a suturally-circumscribed 'patch' appears commonly in the vertical plate of the palatine, simulating an adventitious element in the lateral wall of the nasal fossa. This 'patch' is the maxilla rendered visible from disappearance of the overlying palatine bone. The causative agency in its formation is tooth development, i.e. the progressive increase in size of the more posterior maxillary alveoli.

The attachments of m. semimembranosus. By A. J. E. CAVE and
C. J. PORTEOUS. *St Bartholomew's Hospital Medical College, London*

Certain constant and characteristic features of the attachments of m. semimembranosus are commonly ignored in the descriptive accounts provided by the generality of anatomical treatises: these features are apparent upon nice dissection and may be summarized as follows:

(1) The canonical origin of m. semimembranosus from the superolateral area of the ischial tuberosity is reinforced by an inch-long, falciform, tendinous expansion, taking linear origin from the roughened lateral border of the tuberosity and situate between the origins of m. quadratus femoris anteriorly and m. adductor magnus posteriorly.

(2) The semimembranosus insertion includes a distinct tendinous attachment to a rough tubercle (*tuberculum tendonis*) on the inferolateral aspect of the medial tibial condyle. This slip of attachment derives from the deep aspect of that portion of the general insertion which proceeds over m. popliteus to the oblique line: it is obscured somewhat by the portion which descends over the inferior medial genicular vessels, to gain attachment, by a succession of discrete tendinous slips, to the tibial medial border, contiguous to, and below, the medial lateral ligament of the knee joint.

Repair of mucosal lesions in the rectum of the cat. By R. M. H. McMENN.
(*University of Sheffield*) and F. R. JOHNSON (*London Hospital Medical College*)

In thirty cats, an area of mucous membrane about 1 cm². in size was removed *per anum* from the lower end of the rectum, 2·5 cm. proximal to the muco-cutaneous junction. The animals were allowed to survive after operation for periods ranging from 24 hr. to 6 months; cytological and histochemical studies of the wound areas were then undertaken,

particular attention being paid to the changes in the epithelium and connective tissue in the early stages of the repair process.

Wound healing occurred by migration of epithelial cells from the wound margins, accompanied by an accumulation and maturation of granulation tissue in the floor of the lesion. All the epithelial cells of the crypts, including those at the bases of the crypts, normally gave strong reactions at their free borders for alkaline phosphatase and PAS-positive material. These reactions were not present in cells that were migrating over the floor of the lesion during the first few days. The PAS-positive border was present again by the end of one week, but the reaction for alkaline phosphatase did not return until some days later. Scanty glycogen granules were occasionally present in migrating cells by the end of the first week. The finding of such granules in epithelial cells that did not give a phosphatase reaction, and that formed a lining for shallow depressions which dipped down into maturing granulation tissue, suggested that new crypt formation was occurring. The long-term results after 6 months showed a complete restoration of glands over the whole wound area, although in some earlier specimens a connective tissue septum appeared to be developing between the cut of the mucosa, as described in the mouse by O'Connor (*Brit. J. Surg.* 44, 93, 1956). At all stages of healing, the young connective tissue in the floor of the wound gave a negative reaction for alkaline phosphatase.

The results were compared with studies of wound healing in other organs in the cat.

An instrument for use in the study of the mechanics of the bladder neck. By S. A. VINCENT and G. D. F. MCFADDEN. *Anatomy Department, Queen's University, Belfast; Belfast City Hospital, and Ulster Hospital for Children and Women*

An instrument has been designed to observe and photograph the outlet of the bladder during different phases of filling and emptying.

Measured pressures are produced inside the bladder and measured pressures are applied externally in the region of the bladder neck and urethra. Sutured into the fundus of the bladder is a perspex window with an electric bulb and with two openings, one for filling the bladder with fluid and one for measuring the internal pressure by manometry. Through this window the internal meatus is observed in anaesthetized patients who have got incontinence of urine or urinary obstruction, in isolated fresh post-mortem bladders, and in post-mortem bladders *in situ*. With isolated male bladders antero-posterior pressures applied to the prostate were extremely efficient in stopping outflow but lateral pressures caused the bladder to leak when previously it was holding fluid. The internal meatus could be seen to be forced open by these lateral pressures.

From initial tests with anaesthetized patients the instrument appears to be of some value in diagnosing the type of incontinence or obstruction and therefore in indicating the treatment.

Further results, pressure readings, and photographs are shown and the possibilities of the instrument as an aid to the study of the mechanics of the bladder neck are discussed.

Observations on the fibrous structure of avian bone.
By C. W. M. PRATT. *University of Cambridge*

The femur of the domestic fowl has been examined in embryos and in a series of postnatal stages of known ages. The early perichondrial bone has a different fibrous structure as compared with the early periosteal bone, the fibres in the latter being more irregularly arranged. The periosteal bone found in early postnatal life has a cancellous structure which becomes more compact in older stages. This consolidation is due to the deposition of densely fibred bone within the vascular spaces. As growth slows down the subperiosteal bone contains an increasing amount of longitudinally directed bundles of plaited fibres.

The endochondral bone contains irregularly arranged plaited bundles of fibres. It is at all times confined to the metaphysis and is in part removed with remodelling. In postnatal

stages the metaphysis is demarcated from the diaphysis by a cement line. Endosteal deposition of bone occurs in the diaphysis from the 9th week. At first it is limited to deposits on medullary spongiosa of periosteal origin. Later as growth ceases, endosteal bone is found on all surfaces of the medullary cavity. The fibres of this bone form plaited bundles which are arranged parallel to the surface where the deposition is occurring. In certain situations and particularly in later stages this endosteal bone has a lamellar structure.

Vascular patterns in mammalian striated muscle. By J. E. BARLOW, D. N. WALDER and A. L. HAIGH. *King's College, University of Durham, Newcastle upon Tyne*

It has been suggested by some observers (Barcroft, *J. Physiol.* **117**, 1952, and Lambert, *J. Physiol.* **126**, 1954) that certain vascular phenomena in voluntary muscle can be explained by postulating that arterio-venous short circuiting occurs. This could occur via A.V.A.'s similar to those found elsewhere (Barlow, Bentley & Walder, *Surg. Gynec. Obstet.* **93**, 1951).

A method of differentiating between the arteries and arterioles on the one hand and the capillaries and venous side of the circulatory pattern on the other seems to indicate that A.V.A.'s do not exist in mammalian voluntary muscle.

Red pigment (20% in saline) directly injected into the left ventricle of anaesthetized cats and rabbits circulates till the animal dies. This is followed by injection of 20% blue pigment and 20% rice starch granules in saline into the aorta, which according to Schomer (*Anat. Rec.* **78**, 1940) will not flow through capillaries.

Organs known to contain A.V.A.'s, such as the ear and the stomach, show both arteries and veins blue, but no such mixing has occurred in voluntary muscle, indicating that there has been no extra-capillary passage of material from arterial to venous side.

The mode of termination of sensory nerves and its significance.

By N. CAUNA. *King's College, Newcastle upon Tyne* 1

Human material from 160 individuals from birth to 93 years of age and comparative mammalian material has been studied by cytological and nerve-staining methods, maceration and microdissection.

It has been found that terminal fibres of sensory nerves do not unite. In simple receptors they end in free axoplasmic filaments or terminal condensations, with or without ramification. In complex receptors free endings are uncommon, instead the nerve fibres increase their receptor surface by ramification and by expansion of the terminal fibres into a neurofibrillar apparatus.

Nerve endings are extra-cellular. Endings in epithelia and in terminal corpuscles are intimately attached to the surfaces of the related cells which may belong to the receptor mechanism and play a part in discrimination of sensory modalities. Human hairy and hairless skin is devoid of intra-epidermal nerves, but the epidermis of the nose of certain quadrupeds contains nerves which grow continuously with the epidermis and undergo atrophy on reaching the horny layer where they end either in fading filaments, or in solid condensations, or break into segments.

Axons have a fibrillar structure. The neurofibrillar apparatus is derived from terminal nerve fibres by loss of the axoplasm and expansion of neurofibrils into loose bundles, networks, end bulbs or varicosities. Neurofibrils in the receptors undergo degeneration and are continuously replaced by the expansion and growth of the terminal fibres. Loss and replacement of the neural material in nerve terminals is a physiological process which facilitates the change of pattern of a nerve-ending in its adaptation to changing functional conditions.

**The innervation of the submandibular and sublingual salivary glands of the rat
—a study of cholinesterase in nerves.** By R. S. SNELL. *King's College, London*

The exact mode of termination of the sympathetic and parasympathetic nerve fibres in the vicinity of the secretory units of the salivary glands is still not known. In view of the presence of a higher concentration of cholinesterase in cholinergic nerves than in adrenergic nerves, it was considered that a histochemical study of the cholinesterase in the nerves within the submandibular and sublingual salivary glands of the rat supported by histological studies after degenerative section of one of the two glandular nerves might shed light on the problem.

Forty mature rats were used for the investigation. Frozen sections of the salivary glands, superior cervical sympathetic ganglion, chorda tympani and lingual nerve were processed histochemically. A duration time for incubation with the substrate was determined so that the cholinesterase in the cholinergic pre- and post-ganglionic parasympathetic fibres could be easily detected, leaving the small amount of the enzyme present in the adrenergic post-ganglionic sympathetic fibres undetected. By this means it was found possible to trace the course of the parasympathetic fibres into the salivary glands.

The results showed that the parasympathetic nerve fibres supply the ducts, arteries and all the different secretory units of the submandibular and sublingual salivary glands. The significance of these findings was discussed.

Comparison of nerve structure in the autonomic ground plexus of intestinal muscle as shown by electron microscopy and by silver impregnation. By K. C. RICHARDSON. *University College, London*

In a previous communication it has been established by electron microscopy that the tertiary nerve bundles of Auerbach's plexus and their branches within the muscle coats consist of Schwann cell enveloped bundles of typical non-myelinated nerve fibres. The structural appearance of these bundles under the light microscope following silver impregnation, Champy-Coujard impregnation or methylene blue staining is usually considerably distorted or in some cases incompletely revealed, giving an impression of neurofibrillar networks or of non-specific connective tissue staining. The distinction between interstitial cells and true nervous networks may also be confused. In the case of silver impregnation these distortions appear to be due primarily to poor fixation, rather than to inadequacies in the methods of staining.

If the tonicity of formalin solutions is adjusted with sucrose, and the pH maintained near neutrality during the initial stages of fixation by the addition of small amounts of sodium sulphite in the presence of brom thymol blue indicator, the fixation of rabbit small intestine has been found to be greatly improved. Silver impregnation (of the Bielschowsky-Gros type) will then produce an image of the finest autonomic nerve bundles which is consistent with what we know of their ultra-structure. It is necessary only to use reagents of analytical quality, formalin freshly prepared from paraformaldehyde and ordinary distilled water to achieve a constant and controllable silver impregnation of sufficient quality for the detailed study of experimental material.

An histological, histochemical and electron microscopic study of synovial membrane. By J. D. LEVER and E. H. R. FORD. *University of Cambridge*

Specimens of human, cat and rabbit synovial membrane have been examined (i) by electron microscopy and (ii) by light microscopy in ordinary histological preparations (H and E.), after metachromatic staining and following application of the periodic acid-Schiff technique. Electron micrographs demonstrate that many cells on the synovial surface appear degenerate: these and adjacent deeper cells contain dense granular bodies

approximately $0.05\text{--}0.15\mu$ in diameter. Cells containing larger and metachromatic granules are infrequently seen in the deeper synovial layers: these may be mast cells. A strong PAS reaction is present in and around the cells lining the synovial cavity. These findings suggest that it is the cells on and immediately subjacent to the surface rather than the deeper synovial layers of the mast cells which are responsible for the production of synovial mucin.

Development and structure of the symphysis pubis. By J. J. PRITCHARD
and T. J. HARRISON. *The Queen's University, Belfast*

The structure of the symphysis pubis has been studied in the rat from the 17th day of foetal life to 6 months, and compared with the structure of this joint at various stages in the mouse, guinea-pig, rabbit, dog, macaque and man. In the rat the developing pubic bones become united by a continuous bar of hyaline cartilage just after birth. The lateral parts of the bar serve as growth cartilage; the central part becomes fibro-cartilaginous between the 30th and 50th days. There is no joint cavity and no sexual dimorphism. The angle between the pubic bones opens out by periosteal bony accretion externally and resorption internally.

In the mouse the pubic cartilages do not fuse completely but are partially separated by fibrous, later fibro-cartilaginous, tissue containing a joint cavity which is present in both sexes but is much larger in the female. Joint cavities are also present in the guinea-pig and man, but not in the rabbit or monkey.

Developmental changes in the interventricular foramina. By J. A. SHARP.
University of Leeds

It is commonly stated that the interventricular foramina, at the junction of the third and lateral cerebral ventricles, undergo a relative, rather than an actual, constriction during ontogeny.

Graphic reconstructions of a series of human, pig, rat and rabbit embryo brains, together with wax plate reconstructions of the brains of a similar series of sheep embryos, have permitted accurate measurements to be made of the size of the foramina at different stages of development. The results show that there is undoubtedly active constriction of the interventricular foramina in early embryos of all the species examined.

The factors responsible for this constriction are discussed, with particular reference to a recent suggestion by Fortuyn that the freely projecting caudal poles of the cerebral hemispheres are established by invagination of the diencephalon into the telencephalon.

Subependymal cystic formation during development of the human cerebellum.
By J. D. BOYD. *University of Cambridge*

In late embryonic stages the subependymal zone of the human cerebellum shows multiple small intercellular spaces. By early foetal stages these spaces have become multiple small cysts; these become confluent so that a single large cyst, projecting into the IVth ventricle, comes to be present on each side. Such cysts, which reach their maximum size at about the 45 mm. c.r. length stage, frequently show trabeculation and contain a number of characteristic cells together with occasional blood cells. In later stages the cysts regress and, apparently, disappear completely. It is difficult to suggest a function for the cystic formations; from the present investigations, however, and from observations reported by Hochstetter, it can be stated that they are constantly present. Their eventual disappearance may possibly be associated with the so-called 'eversion' of the cerebellum which occurs at about the time when the cysts regress.

The effect of various solutions upon nodal and internodal diameters of living nerve fibres. By B. HIGGS. *Royal Free Hospital School of Medicine, London*

Fresh teased preparations of rat sciatic nerve were examined under a phase-contrast microscope and photographed before and after the application of 10% formalin or Bouin's solution, hyper- or hypotonic Tyrode. Axonal diameters were measured in the nodal and inter-nodal regions. Statistical analysis showed a significant decrease in nodal but not in the inter-nodal regions after addition of fixatives, suggesting a greater permeability in the nodal region. A significant decrease and increase of nodal axon diameter is obtained with hyper- and hypotonic Tyrode respectively, suggesting the existence of a semi-permeable membrane at this site. Some of the axon shrinkage due to fixation might be attributed to passage of water from the axon in response to the high osmotic pressure of the fixative. It has been suggested that extensive axonal narrowing seen in fixed preparations at the nodes of Ranvier was a fixation artefact, since it was not seen in living nerve fibres. Some limited narrowing may exist behind the myelin at the node but this could not be determined.

The effect of testosterone and orchidectomy on the activity of the melanocytes in the skin. By P. G. BISCHITZ and R. S. SNELL. *King's College, London*

The relationship between the sex hormones and the activity of the melanocytes is not clearly understood. The present research was designed to investigate histochemically the effect of testosterone and orchidectomy on the activity of the melanocyte in the skin.

Skin biopsies were taken from eighteen mature guinea-pigs from the ear, anterior abdominal wall and sole of foot. In addition, the areola and nipple were excised on one side. The animals were divided into three groups (1) Animals were orchidectomized and left untreated for 4 weeks. (2) Animals were orchidectomized and 1 month later a 4-week course of testosterone injections given intramuscularly was commenced. (3) Animals were left intact and were given a course of testosterone identical to that received by group 2. After the animals had been killed a further series of skin specimens were removed from areas adjacent to those taken previously and the remaining areola and nipple were excised. Full thickness and split skin specimens were then processed with the Dopa reagent.

The results showed that in all groups of animals no uniform changes were produced in the activity of the melanocytes in the skin of the ear, anterior abdominal wall and areola. In the sole of the foot all three groups of animals showed an increased amount of melanin in the basal layer of the epidermis. The significance of these findings was discussed.

The form of the aortic arches in rat embryos at different stages of development. By D. B. MOFFAT. *University College, Cardiff*

The aortic arch system has been studied in a series of rat embryos of different ages, dissected after the injection of Indian ink into the vascular system.

There are two points of particular interest in the rat. Firstly, in embryos having C.R. lengths of between 7 and 9 mm., the fourth arch has a marked caudally directed convexity which brings it into contact with the sixth arch. Very frequently, embryos are found which have a communication between the fourth and the sixth arches at the point of contact, and it is probable that this is a constant feature of development.

Secondly, at one stage, the pro-atlantal artery takes origin from the ductus caroticus, which enlarges slightly. It is not until the pro-atlantal artery reaches the level of the third aortic arch that the ductus caroticus disappears.

Other less important points are described, and the results discussed in relation to a previous paper on the direction of blood flow through the aortic arches.

The protective effect of insulin in relation to the teratogenic activity of cortisone and hypervitaminosis-A. By J. W. MILLEN and D. H. M. WOOLLAM. *University of Cambridge*

In a previous communication it was reported that the administration of cortisone to female rats during pregnancy enhanced the teratogenic action of large doses of vitamin A. Further experiments have been carried out to study the effect of insulin on the incidence of malformations of the brain and palate produced by hypervitaminosis-A and by cortisone and hypervitaminosis-A in combination.

Female rats of the Wistar Strain were used in the experiments. Pregnancy was determined by the presence of spermatozoa in the vaginal smear. The pregnant rats were divided into groups of twelve. Vitamin A dissolved in arachis oil was given by intubation: cortisone acetate and insulin were injected subcutaneously. The animals were killed on the 20th day of pregnancy and the young were removed from the uterus for examination.

The results of this work suggested that insulin exerted a protective effect against the potentiating action of cortisone in relation to malformations produced by material hypervitaminosis-A. It appeared also that insulin may have some moderating influence on the teratogenic activity of large doses of vitamin A given to female rats during pregnancy.

Influence of cortisone on teratogenic effects of radiation. By D. H. M. WOOLLAM, J. W. MILLEN and J. A. F. FOZZARD. *University of Cambridge*

It has been shown that the administration of cortisone greatly increases the incidence of deformities of the brain and palate in the young of female rats subjected to experimental hypervitaminosis-A (Millen & Woollam, *Brit. med. J.* 2, 1957; Woollam & Millen, *Brit. med. J.* 2, 1957). The present report deals with the effects of cortisone on the incidence of eye defects in the young of female rats subjected to X-radiation during pregnancy.

Pregnant rats of the Wistar strain were divided into three groups, each group consisting of twelve animals. Animals in groups 1 and 2 received 258 r. X-radiation to the anterior abdominal wall on day 11 of pregnancy. Animals in groups 2 and 3 received 20 mg. cortisone acetate by subcutaneous injection daily from day 9 to day 12 of pregnancy. The rats were killed on day 20 and the young removed for examination.

None of the young from animals belonging to group 3 (cortisone only) showed any abnormality. Defects of the eye ranging from anophthalmia to coloboma were found in both group 1 (radiation only) and group 2 (radiation and cortisone). The incidence of the deformity was 18.8% in group 2 and 5.2% in group 1.

The findings suggest that cortisone augments the teratogenic activity of radiation. The significance of this observation is discussed.

Dorsal mid-line defects in two human embryos. By W. R. M. MORTON. *The Queen's University, Belfast*

Two human embryos showing rarely reported abnormalities are described. The first, an embryo of about 6 mm. c.r. length (Streeter's age group XIV) shows duplication of the upper cervical part of the notochord with abnormal development of the overlying central nervous system, and additional somites on the left side. A small ectodermal cyst adjacent to the hind brain would appear to be a duplication of the otocyst. The second embryo, of about 30 mm. length, shows the typical facies of a full-term anencephalic foetus. The dorsal part of the brain is absent and its remnants protrude through the defective occiput. The spinal column is bifid from the occiput to the coccyx but the medullary plate is covered by surface ectoderm in the sacral and coccygeal regions. Such extensive defects are rarely seen at the embryonic stage. The possible significance of these defects, which resemble some of those produced experimentally in animals by other workers, is discussed.

The development of the coverings of the testis and cord. By K. M. BACKHOUSE
(*Charing Cross Hospital Medical School, London*) and H. BUTLER (*University of Khartoum*)

The concept of the gubernaculum testis as a mesenchymatous residuum around which the body-wall structures develop is incompatible with the generally accepted theory of the development of the coverings of the testis and cord.

In pig, sheep and man the cremaster muscle develops in the mesenchyme of the gubernaculum testis, and the extent of its development is closely related to that of the processus vaginalis. The cremaster muscle develops within the extra-vaginal portion of the gubernaculum, which retains its continuity with the body-wall mesenchyme and connective tissue around the inguinal canal, after the more distal portions of the gubernaculum have become separated from the superficial fasciae of the abdominal and scrotal walls.

Following testicular descent the cremaster muscle gains its inferior insertion into the root of the mesorchium. The extravaginal portion of the gubernaculum testis, in which the cremaster muscle has developed, becomes differentiated into connective tissue forming fascial sheets superficial and deep to the cremaster muscle, the external and internal spermatic fasciae. These fascial sheets are, consequent upon their development, continuous with the deeper fasciae of the abdominal wall.

Neither the cremaster muscle nor the spermatic fasciae can be considered as homologous with the layers of the anterior abdominal wall but as derivatives of the gubernaculum.

FEBRUARY 1958

An ordinary meeting of the Society for the Session 1957-8 was held on Friday, 28 February 1958, in the Department of Anatomy, The Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C. 2. The President (Prof. F. GOLDBY) was in the Chair.

The following are the authors' abstracts of the papers presented.

Human cardiac veins. By R. J. LAST. *Royal College of Surgeons, London*

Injections of synthetic resin into the right side of the heart, followed by maceration, reveal a rather constant pattern of cardiac veins not entirely consistent with conventional descriptions. In particular, attention is drawn to the list of names (p. 36) under *Venae cordis* in the 'Nomina Anatomica' submitted to the Sixth International Congress of Anatomists in Paris in 1955. 'V. posterior ventriculi sinistri' is not often single but is usually represented by two veins of equal size. If 'V. cordis parva' in this list is the companion vein to the marginal branch of the right coronary artery, it should be noted that it opens directly into the right atrium with the other anterior cardiac veins, and does not join the coronary sinus, as commonly stated.

The effect of water deprivation on the neurosecretory material of the desert rat (*Meriones meriones*). By A. HOWE (*Royal College of Surgeons, London*) and P. A. JEWELL (*Royal Veterinary College, London*)

Anatomical observations on the relative size and structure of the neural lobe and the hypothalamo-hypophysial neurosecretory system of rodents have been published (Hansson, 1956, *K. fysiogr. Sällsk. Lund. Handl.*, N.F. 67, Nr. 16 and 17). The present observations compared responses of these structures to water deprivation in a desert-living rodent (*Meriones meriones*) with those in laboratory rats.

Experimental animals were maintained on a dry diet (approx. 12% water content); controls had, in addition, free access to water. On the 10th day the animals were killed and the whole brain fixed by arterial perfusion with Helly's fluid. Serial frontal sections

(8 μ) of hypothalamus and pituitary were stained for 'neurosecretory material' (NSM) by the chrome alum-haematoxylin method. Water deprivation resulted in laboratory rats losing some 35 %, and desert rats 17 %, of their initial body weight. Accompanying this loss was a virtually complete depletion of NSM from the pars nervosa of laboratory rats; histological appearances of pars nervosa of desert rats, however, remained unaffected. The amount of NSM in the hypothalamus of normal animals was variable, but it appeared that the desert rat showed relatively less NSM in the hypothalamus after water deprivation than did the laboratory rat under the same conditions.

Estimation of deoxyribonucleic acid in ganglion cells by microphotometry of Feulgen-stained sections. By C. J. STRATMANN. *The Royal College of Surgeons, London*

Biochemical methods have shown substantial changes in nucleic acid content of ganglion cells during chromatolysis and after stimulation. In an attempt to define more accurately the site of these changes, direct cytochemical estimations have been carried out on the nuclei of the superior cervical ganglion of the rabbit.

Feulgen-staining of sections, followed by microphotometric estimation of intensity of staining of nuclei, is well established as a reliable method for estimation of deoxyribonucleic acid in nuclei. This method has been applied to sections of superior cervical ganglion and it has been found that reproducible results could be obtained when all stages of the staining technique are rigorously controlled.

Absorption values indicate that deoxyribonucleic acid content of neurone nuclei is constant between narrow limits at different levels of the ganglia, between opposite sides of the same animal and between different animals. Changes in this level following post-ganglionic section are reported and discussed.

The effect of ovariectomy, oestrogen and progesterone on the activity of the melanocytes in the skin. By P. G. BISCHITZ and R. S. SNELL. *King's College, London*

A histochemical investigation into effects of ovariectomy, oestradiol benzoate and progesterone on activity of melanocytes in skin was carried out on eighteen mature female guinea-pigs.

Skin specimens were removed from six animals before and 1 month after ovariectomy. The twelve remaining animals were ovariectomized and 1 month later skin biopsies were taken. Six of these animals then received a 4-week course of oestrogen injections and the remaining six received a similar course of progesterone. After the animals were killed a further series of skin specimens was removed from areas adjacent to those taken previously. All the skin specimens were processed with the Dopa reagent.

After ovariectomy many melanocytes in skin of ear, anterior abdominal wall and areola contained less melanin and the cell bodies and processes of some melanocytes appeared shrunken. The oestrogen injections resulted in an increased amount of melanin both within and outside the melanocytes but their shrunken appearance remained unchanged. Progesterone was seen to have a similar effect on melanogenesis to that produced by oestrogen but the changes were less marked. The significance of these findings was discussed.

Observations on chondrogenesis in a foetal long bone. By C. W. M. PRATT.
University of Cambridge

The first indication of the femur in the hind-limb skeletal blastema of the rat foetus is the centre of chondrification, which in a longitudinal section appears as a mass of closely packed and irregularly arranged cells. The surrounding blastemal cells become elongated and orientated with their long axes forming concentric layers. These cells are separated

from adjacent skeletal elements by unorganized interzones. Matrix appears in the centre of chondrification and amongst those orientated blastemal cells which lie between the centre of chondrification and the interzones. The diaphysial cartilage thus formed is limited by a longitudinally directed fibrous sheath separating it from the surrounding orientated blastemal cells, and these cells now become the perichondrium. The epiphysial cartilage is formed from the interzone. The interzone becomes divided, and after differentiation of the articular surface the chondrogenic layer of the femoral epiphysis gradually disappears. The subsequent development of the epiphysis, and the components of the diaphysial cartilage shows differences which reflect the earlier development.

The development of the sinus venosus type of atrial septal defect.

By H. R. S. HARLEY. *Sully Hospital, Glamorgan*

No satisfactory explanation for the development of the high or sinus venosus type of atrial septal defect has yet been given.

It is suggested that this defect arises because the sinuatrial orifice does not shift as far to the right as it should do. This means that the septum primum lies against the left venosus valve, that no interseptovalvular space develops, and that the cephalo-dorsal limb of septum secundum cannot form by inflection of the atrial wall at this site. This means that a deficiency occurs in the attachment of septum secundum facing the superior vena caval orifice. Because this deficiency lies opposite the ostium secundum an interatrial defect occurs.

The junction of cerebral hemisphere and third ventricle in mammalian embryos.

By J. A. SHARP. *University of Leeds*

The area over which the medial wall of the cerebral hemisphere is attached to, and continuous with, the lateral wall of the third ventricle has been examined by means of graphic reconstructions based on embryos of man, pig, rat and rabbit.

For descriptive purposes, this area may be divided into four regions, lying respectively dorsal, rostral, ventral and caudal to the interventricular foramen. By extension of a term originally used by His, the whole area may be referred to as the 'pedicle area'.

The caudal region has been studied quantitatively and the rate of growth of this portion of the pedicle area, corresponding to the posterior wall of the interventricular foramen, has been found to increase rapidly when fibres of the internal capsule begin to grow through it.

It is suggested that these measurements may throw some light on the question of occurrence of fusion between the medial hemisphere wall and the lateral wall of the diencephalon during formation of the internal capsule.

A new method of graphic reconstruction.

By D. M. POTTS. *University of Cambridge*

A movable drawing-board has been designed for making graphic reconstructions directly onto graph paper in one step. The apparatus is orientated with respect to each section in the series, and as the board is slid beneath the projected images the outlines of the features being drawn can be reproduced directly upon graph paper fastened to the board. The new method is at once more accurate and considerably faster than the traditional one.

Early embryology of the nephric system in the sheep.

By B. TOWERS. *University of Cambridge*

Examination of serial sections of closely graded sheep embryos with fewer than twenty-five somites has yielded observations difficult to interpret on the basis of the theory, current during the past 60 years, that the mammalian nephric system is exclusively of mesodermal

origin. A 'renal line' of dorsal ectoblast starts at the side of the caudal end of the primitive streak (where it is in contact with endoderm of the cloaca and marks the site of entry of the future Wolffian Duct) and extends as far forwards as the 7th somite. Throughout most of its length this renal line appears to give rise to the primordium of the Wolffian Duct by a simple process of proliferation and delamination, after which the duct primordium makes contact, apparently secondarily, with intermediate-mesoderm. In its most cranial portion there occurs more intimate fusion between ectoderm and mesoderm, and here too ectoblast cells play a significant role in nephric development. Reference is made to observations of nineteenth-century workers such as His, Hensen, Graf Spee, Flemming, Meyer and Kollmann in support of the present findings.

The phylogeny of the labyrinthine receptors.

By C. C. D. SHUTE. *University of Cambridge*

de Burlet's identification (1930) of the subdivisions of the inner ear in lampreys is rejected. The chamber regarded by him as the saccule is absent in larval stages, and appears to be a late derivative of the utricle. Its end-organ with that of the so-called dorsal endolympathic duct shows certain affinities with the lateral crista of higher forms. On account of its innervation and relationships, de Burlet's lagena is renamed the saccule: its macula is bilobed, but has no distinct lagenar portion. Behind it, the macula neglecta occupies a similar position to that of the utricular macula in the pars anterior of the labyrinth.

This interpretation is supported by the observation, made on reptilian embryos, that the primordial papilla (macula) neglecta develops between the saccular macula and the posterior crista. It is shown how a portion of the papilla neglecta could have given rise to one of the main hearing organs of Amphibia—the papilla amphibiorum. Rarely in mammals does a vestigial papilla neglecta persist within the ampulla of the posterior semi-circular duct. This 'crista quarta' (Benjamins, 1913), although mentioned in human anatomical text-books is not commonly present in man.

The nasal aperture in animals and Man. By Sir VICTOR NEGUS with the collaboration of T. V. L. CRICHLow. *Royal College of Surgeons, London*

The communication deals with the available airway through the nose. Consideration is directed (*a*) to the position, size and shape of the anterior nares, and the degree of dilatability; (*b*) to the size of the anterior bony aperture; and (*c*) to the available space for passage of air through the nasal fossae, as determined by the relative size of the maxillo and ethmo turbinal bodies. The relation of available nasal airway to variations of climate is discussed.

On the anatomy and development of the nasal cavity in the gannet (*Sula bassana, L.*) By R. J. SCOTHORNE. *University of Glasgow*

Among a number of structural specializations in gannets, one of the most interesting is an extreme modification of the nasal cavity. In adult birds, the external nares are completely occluded, the vestibular portion of the nasal cavity is virtually obliterated, and nasal conchae are absent. Despite occlusion of the external nares and vestibule and the consequent absence of any free flow of air through the nasal cavity, the olfactory epithelium and the olfactory nerves are well developed. Moreover, the nasal glands are relatively large.

In newly hatched gannet chicks the bony external nares are large, but are occluded by the rhamphotheca. Early stages in development of the nasal cavity are essentially similar to those seen in other species studied—*Larus*, *Gallinula*, *Turdus* and *Columba*. An olfactory pit forms in the usual manner and the external nares and vestibular portion of the nasal cavity are soon occluded by an epithelial plug. This plug breaks down in the latter half of incubation in all except gannets, in which it becomes progressively more attenuated with the growth of the bill. In late gannet embryos a slender epithelial strand may be traced from the occluded external naris to the definitive nasal cavity.

The maxillary nerve in primates. By E. H. ASHTON and C. E. OXNARD.
University of Birmingham

Corresponding branches of the maxillary nerve are found in each of the main subgroups of the order Primates, such variations as are encountered affecting mainly the proportions of nasal and labial divisions of the infraorbital nerve. These differences can be correlated with development of a rhinarium and with variations in complexity of facial muscles.

Thus, in the tree shrew, lemur, loris and galago, where a rhinarium is associated with an uncomplicated facial musculature, the nasal branch is the bigger, and relatively few fibres of the labial branch are fasciculated with twigs of the seventh nerve. In New and Old World monkeys where there is no rhinarium, but where facial muscles are more highly developed than in prosimians, the labial nerve is normally the bigger and more of its fibres are associated with the seventh nerve. In apes where the facial muscles are more complex than in monkeys, still more fibres of the labial branch join with twigs of the facial nerve, while in man, the trend continues further, and the infraorbital plexus is more extensive than in any lower primate.

Denervation experiments on the palatal mucosa.
By A. D. DIXON. *University of Manchester*

Degenerative changes which follow nerve division confirm a previous observation that the nature of nerve fibres in oral nerve plexuses can be demonstrated by the Champy Osmium tetroxide method. Thirty-six animals—monkeys and rabbits—were subjected to various operative procedures involving interruption of the somatic nerve supply to the palatal mucosa and/or removal of part of the cervical sympathetic trunks.

Avulsion or cauterization of somatic sensory nerves at appropriate points produces degeneration of myelinated fibres within eight days of operation. In animals allowed to survive for periods varying from 7 hr. to 100 days after unilateral or bilateral cervical sympathectomy fine beaded nerve fibres, as revealed by the Champy technique, become granular and ultimately disappear.

In unilateral cervical sympathectomy some of these fibres persist on the homolateral side. These may be accounted for by fibres passing in alternative pathways via intermediate ganglia, additional sympathetic trunks, vascular plexuses, overlap of sympathetic fibres in the mid-line of the palate and existence of parasympathetic fibres.

The use of serial shadowed replicas in the optical and electron microscopic examination of the hard structures of a tooth as displayed by progressive etching. By C. H. TONGE and E. H. BOULT. *King's College, Newcastle upon Tyne*

Human teeth of known history have been bisected. One half has been prepared as an ordinary ground section and serves as a control. The other half has been polished and examined by means of shadowed optical and electron microscope replicas which have been prepared using a modification of a standard technique (Scott & Wyckoff, *Publ. Hlth Rep.* 62, 1947). Selected areas of preparations from the polished but unetched surface have been examined by the optical and electron microscope. Microdissection of the tooth surface has been carried out by means of progressive etching. Serial replicas have been made after each etch and a further study of the same selected areas carried out. Several replicas of each stage have been made with different metal-shadowing directions. This technique enables the position and arrangement of individual structures to be ascertained optically prior to examination of similar fields with an electron microscope. Structures can be identified at successive stages as a result of progressive etching and the matrix of dental tissues revealed. In particular, the position of cementum lacunae can be demonstrated together with the arrangement of the component fibres of its matrix in which collagen can be identified by means of the electron microscope replicas.

A combined light- and electron-microscope study of the normal, hypertrophic and activated rat parathyroid. By J. D. LEVER. *University of Cambridge*

Unilateral parathyroidectomy preceded all experiments and provided normal control glands. The remaining parathyroids were then removed: (i) after 7 weeks; (ii) following bilateral nephrectomy; (iii) after phosphate administration. Glands were: (a) stained to demonstrate cytoplasmic nucleic acids; (b) examined by electron microscopy; and (c) serially sectioned and stained with haematoxylin and eosin. Cell counts per unit area and estimations of gland mass were made from the serially sectioned material.

A range of normal cytological variations was observed: experimental results were interpreted in the light of such variations and having regard to any change in cell size. A simple compensatory hypertrophy of the remaining gland followed unilateral parathyroidectomy. In the parathyroid activated by phosphate administration or bilateral nephrectomy the majority of cells showed an increase in size, an absolute increase in cytoplasmic RNA and an expanded endoplasmic reticulum.

The serial sectioning of undecalcified animal heads. By P. A. ROBERTS
and D. A. N. HOYTE. *University of Manchester*

Serial sections have been made of whole heads of young animals to show intravitally stained bone in its natural relationships.

The fixed and dehydrated heads are infiltrated with and blocked in Ester wax, up to 3-4 weeks being required for the largest specimens. These have been cut in a parasagittal plane to facilitate infiltration.

Sectioning is carried out on a heavy-base sledge microtome using a specially hard knife. A strip of 'Sellotape' is firmly pressed onto the surface of the block (Duthie, *J. Path. Bact.* **68**, 1954) and on cutting the section remains adherent to the tape. Sections of 10μ upwards have been obtained.

Two methods have been used in mounting. In one, the sections on the tape are dewaxed in ethylene glycol 1-butyl ether and mounted, still on the tape, face downwards in balsam. These sections are unstained except for intravital alizarin red. In the other, the sections are mounted on dry albumen-coated slides on a hot-plate, and the tape removed by 24 hr. immersion in Xylol. Fresh Xylol completes dewaxing, and sections can then be treated in the usual way prior to staining.

Unfixed sections of peripheral nerve. By P. L. WILLIAMS.
Guy's Hospital Medical School, London

Methods used in quantitative structural analysis of peripheral nerve should provide information that reflects closely the natural state. Alternatively, any distortion inherent in a method should be subjected to a critical evaluation, and further, different methods should have a firm basis for comparison.

A technique for producing 5μ sections of fresh unfixed nerve has been evolved. The nerve, implanted in supporting tissue, is placed in a closed glass cell and rapidly frozen in solid CO_2 -acetone mixture. Microtomy and attachment to the slide are performed in a cryostat at -20°C . The sections are immersed in saline, examined directly using various forms of illumination, and photographed. Subsequent silver impregnation or myelin staining procedures may be carried out.

A rapid freezing technique is probably the best method available for producing sections with minimal distortion. Further, the technique compares favourably with routine frozen sectioning—serial sections may be examined, 5μ sections allow more critical photography, and complete sections of nerve trunks may be obtained.

The method is being used to study the ratio between axonal and external fibre diameters, myelinated fibre diameter size-frequency distributions, the paranodal contours of myelin sheaths and a comparison of orthodox fixation and staining procedures applied to nerve fibres.

The size of the axon and its myelin sheath. By P. L. WILLIAMS and
C. P. WENDELL-SMITH. *Guy's Hospital Medical School, London*

Various mathematical functions of the diameter of peripheral nerve fibres have been related to conduction velocity. It has also been suggested that a closer correlation may exist between the velocity and the axonal rather than the external diameter. The ratio 'g' (axonal/external diameter) has been investigated by several observers with varying results. The preparations used have included stained sections of fixed material, and teased preparations and frozen sections, 15–20 μ thick, of fresh nerve. These methods, the results obtained, and some of the problems involved have been discussed.

A reassessment of the ratio 'g' for mammalian myelinated nerve fibres has been made. Unfixed transverse sections of 5 μ thickness have been prepared. Measurements were taken from photographs enlarged to 1000 diameters and also made directly on the fresh preparation using a screw ocular micrometer.

A graph illustrating the relationship of the ratio 'g' to external fibre diameter has been constructed. The resulting curve differs in several respects from those presented by other observers. The possible implications of this have been discussed.

Further observations on the morphology of the myenteric plexus.
By J. R. RINTOUL. *University of Manchester*

In a previous communication it was demonstrated that the patterns of the myenteric intestinal plexuses from a variety of vertebrates was characteristic for each animal, the pattern varying from the simple uniform arrangement seen in reptiles and birds to the complex form seen in mammals.

The form of the myenteric plexuses also varied in different regions of the alimentary tract. Little change in the patterns was evident in preparations made from different regions of the gut in the reptiles and birds studied, but there was a marked change in the form of the meshwork in mammals.

The portions of the gut studied were removed from the animal immediately after death, threaded on glass rods of suitable diameter and kept in position thereon until fixation and staining were completed; this obviated any possible alteration to the patterns of the myenteric plexuses due to uneven shrinkage or stretching of the tissue.

Photomicrographs of the preparations were shown and described.

Auerbach's plexus in the rabbit. By R. E. COUPLAND
and R. L. HOLMES. *University of Leeds*

Histological and histochemical methods have been used including routine stains, silver impregnations, methylene blue, PAS and histochemical methods for the demonstration of cholinesterase, non-specific esterase, acid and alkaline phosphatase and peroxidase.

The nerve fibres are enclosed in well-defined cellular tubes whose outer walls are composed of reticular fibres. Transversely disposed nuclei are a constant feature of these structures and lie centrally. They appear to be non-nervous in nature. Primary, secondary and tertiary strands of the plexus are well shown by both methylene blue and methods for localization of cholinesterase.

The interstitial cells of Cajal have been demonstrated by both silver and methylene blue techniques but have a negative cholinesterase reaction. The processes of these cells run along the surface of the cellular strands of the plexus where they are in contact with reticular fibres but have not been observed in close association with neurones.

A positive reaction for true and pseudocholinesterase is given by both the nervous and supporting elements of the plexus.

Foregut diverticulum with spina bifida and diastematomyelia.By H. E. ROBSON. *University of Birmingham*

A still-born 7-month foetus with multiple congenital abnormalities was described. A diverticulum arising from the greater curvature of the stomach is associated with an extensive anterior spina bifida of the cervical and most of the thoracic vertebrae. The cleft atlas articulates with the skull in the mastoid region. The cervical and upper thoracic regions of the spinal cord are also split, and lie on either side of the gastric diverticulum, which has penetrated into the skull through the vertebral cleft and a defect in the occiput.

Fixation of the gastric diverticulum has prevented rotation of the gut. The lesser curvature of the stomach lies anteriorly, the spleen is in the midline, and the whole intestine suspended from a single midline dorsal mesentery. The diaphragm has also been prevented from descending posteriorly, and lies in an almost vertical plane, separating the heart and lungs anteriorly from the stomach posteriorly.

Hare lip, cleft palate and coarctation of the aorta are also present. Possible embryological explanations of the findings are suggested.

The curvature of the occipital bone in Bushmen and Negroes. By P. V. TOBIAS
(read by R. DART). *University of the Witwatersrand*

There are at least two distinct patterns of occipital curvature among Africans, each of which is extreme for living races.

Bushmen, like all fossil men up to Late Pleistocene times, have strongly-curved or bun-shaped occipitals. The degree of curvature may be expressed as a percentage ratio of the lambda-opisthion chord and arc. The bigger this occipital chord-arc index, the flatter the occipital, and vice versa. The mean value for 152 male Bushmen crania is 81.2 and for 100 female crania 82.2: these are the lowest mean values recorded for any living race (cf. Rhodesian Man, 75.2; Kanjera I, 78.8; Singa, 77.2; Gamble's Cave IV, 77.7; Mechta I, 80.8).

On the other hand, South African Bantu-speaking negroids, like West African Negroes, have flat occipitals:

Natal Nguni (Zulus and Swazis)	85.6 (158 males)	84.5 (45 females)
Cape Nguni (Xosa, Fingo, etc.)	84.1 (120 males)	84.1 (33 females)
Cameroons Negroes	86.9 (82 males)	
Ashanti	85.5 (40 males)	
Congo Tetela (Western Bantu)	87.9 (36 males)	

As compared with Bushman and fossil crania, negroids have an occipital which is not only flatter, but hinged downwards on the lambdoid suture; the parietal, conversely, is more highly arched. The presumed rearrangement of brain substance which makes the Negro's parieto-occipital contour possible is a late development in human evolution: it accompanies and is doubtless responsible for the earliest appearance of brachycephaly in man.

Growth changes in the occipital bone of Bushmen and Negroids.
By P. V. TOBIAS (read by R. DART). *University of the Witwatersrand*

The divergence between the highly curved occipital bone of adult Bushmen and the flattened occiput of adult Negroids signifies two different patterns of occipital growth. An attempt has been made to ascertain how early in development this difference becomes established.

From a study of 60 crania of non-adult Bushmen and Bantu-speaking Negroids, it is concluded that child crania have prominent occipital bulges, in contrast with Pearson's view, based on inadequate samples, that non-adults have flat occipitals (*Biometrika*, 16, 1924).

Until the first permanent molars erupt, both Bush and Bantu crania have marked occipital curves. Beyond that age, Bantu occipitals hinge downwards at the lambdoid suture and flatten out, progressively approaching the adult contour.

Bush crania are not subject to the same flattening or hinge-movement, and retain a highly arched occipital through to adulthood. This infantile feature is one example amongst many of infantile morphological traits in adults of the Bush race. It has been suggested elsewhere that such 'pedomorphism' results from retarding genetic mutations which slow up the rates of differentiative processes; or that the converse traits in the Negro follow accelerating mutations. Perhaps the divergence of Bantu from Bush patterns of occipital growth about the time the permanent teeth start erupting, suggests the time of effective operation of some of these accelerating or retarding morphogenetic influences.

APRIL 1958

An ordinary meeting of the Society for the Session 1957-8 was held on Friday and Saturday, 25 and 26 April 1958, at the Zoological Society of London, Regent's Park, N.W. 1. The President (Prof. F. GOLDBY) and Vice-president (Prof. R. WARWICK) occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers presented:

The medial inclination of the thoracic intervertebral articular facets in relation to axial rotation. By P. R. DAVIS. *Royal Free Hospital School of Medicine, London*

The intervertebral articular facets of a given thoracic vertebra have been described as forming arcs of a circle whose centre lies within the boundaries of the vertebral body, thus permitting and directing rotatory movements. Others have thought that, if the facets do lie on the circumference of one circle, that circle has its centre in front of the vertebral body.

In the present series of observations, measurement of the angle between the chords formed by pairs of facets has proved of little value, but determination of the position of the point of intersection of the lines bisecting the chords has revealed a fairly constant pattern in different vertebral columns. Assuming that each pair of facets lies on the circumference of one circle, then in the upper thoracic vertebrae the centres of the circles are in front of the vertebral bodies; in the mid-thoracic series they approach and frequently lie within the line of the bodies; in the lower thorax the centres most commonly again lie in front of the vertebral bodies.

The findings are discussed in relation to axial rotation of the thoracic vertebrae at different levels.

The sex of isolated femora. By F. L. D. STEEL. *The London Hospital Medical College*

Standard osteometric measurements have been made on 108 adult human femora of known age and sex; from these, three (maximum length, bicondylar breadth, maximum transverse diameter of head) have been chosen for combination into a function of the type $X = a_1x_1 + a_2x_2 + a_3x_3$.

The measurements used could be applied simultaneously (owing to postmortem damage) to only seventy-one bones.

The value of the discriminant is $X = 0.0000073275x_1 + 0.0012646731x_2 + 0.003939753x_3$, and its use allows a within-sample accuracy of 90% in allotting to each bone its sex correctly.

The values for the 'typical male' and 'typical female' bones are $X = 0.292$ and $X = 0.258$, respectively. $X_m = 0.291$, s.d. 0.017 and $X_f = 0.259$, s.d. = 0.010. 't' for discriminant is significant at $P = 0.01$.

An experimental study of the temporal ridges in the rat.By D. DARLINGTON. *University of Birmingham*

During the early development of the rat the temporal muscles and ridges extend upwards on the side of the cranium. It is likely therefore that the final position of the ridge is related to the eventual degree of development of the muscle. The hypothesis that the distance between the temporal ridges of the rat is inversely proportional to the size of the temporal muscles has been tested experimentally.

Forty-five pairs of litter-mate weanling rats were used. One animal in each pair was reared on a hard cube diet and the other on the same diet finely ground and mixed with water. The rats were killed when they were about twenty weeks old.

The temporal muscles were heavier in the animals fed the hard cube diet, but there was no evidence that the temporal ridges were any closer together in these animals than in those fed soft mash. On the other hand, the ridges were thicker and higher in the rats fed the harder diet.

The relative contribution of sutural and of ectocranial deposition of bone to cranial growth in rodents. By D. A. N. HOYTE. *University of Manchester*

The prevailing idea of cranial growth is that it takes place rapidly after birth by separation of the sutures, with bone replacing membrane in the vault (Massler & Schour, *Anat. Rec.* **110**, 1951) and replacing cartilage in the base.

In observations on new-born rodents injected intra-vitally with alizarin red, this is seen to be only partially true. The degree of sutural growth has been overestimated by false measurements from impermanent alizarin lines. There is over-all ectocranial deposition in the vault and base, except over the parietal and frontal eminences (where stasis or even resorption may be seen), or in some parts of the orbito-temporal fossae. In the vault, since this ectocranial deposition is greatest towards the bone margins, it provides both for enlargement of the cranium and for alteration of bone curvature. It is combined with endocranial resorption of bone, seen in lesser degree also over the bones of the base. The endocranial deposition seen in older animals leads to thickening of the bones, and cannot be a means of increasing the cranial capacity.

These observations confirm and extend those of Brash (*Edin. med. J.* **41**, 1934).

Skull growth in the Rhesus monkey (*Macaca mulatta*). By R. A. LATHAM.
The Queen's University, Belfast

Seven monkeys between the ages of $1\frac{1}{2}$ and $4\frac{1}{2}$ years were used. Growth sites were determined from a comparison of alizarin-stained surfaces soon after injection with those after sufficient time had elapsed for new bone to have been deposited on those surfaces.

Confirmation was obtained from the measurement of selected skull parameters at different ages, and from the histological examination of certain sutures and synchondroses.

In the period studied the sites of maximal growth were found to be:

(1) the inner surface of the cranial vault; (2) the basal synchondroses; (3) the condyle, posterior border and sigmoid notch of the mandible; (4) the lateral surface of the zygomatic arch; (5) the facial margin of the orbital cavity. The hard palate showed accretion on its undersurface and resorption on its upper surface. The significance of the persistence throughout the growing period of an active mid-sphenoidal synchondrosis in the monkey has been generally overlooked: it evidently plays a dominant role in the development of simian prognathism.

The blood supply of the rat's femur in relation to the repair of cortical defects.By G. O. McAULEY. *The Queen's University, Belfast*

The normal blood supply of the rat's femur, and the effects of partial deprivation of the blood supply on the repair of a cortical defect, were studied. Specimens for gross examination were injected with 'Micropaque' or 'Neoprene'; sections were studied after injection with Indian ink.

Interruption of the metaphyseal and nutrient vessels led to wholesale necrosis of the cortex and marrow. The cortical defect was then invaded by granulation tissue from outside the periosteum, while new bone and some cartilage were laid down under the fibrous periosteum. Later, this new bone spread into the granulation tissue within the defect.

Interruption of the nutrient and periosteal vessels, with isolation of the shaft by means of a polythene sheath, likewise produced complete cortical and medullary necrosis except at the metaphyseal extremities. Granulation tissue followed by cancellous bone spread from the surviving marrow at the metaphyses into the central necrotic marrow. Externally, starting from either end, granulation tissue and cancellous bone advanced along the shaft both outside and inside the polythene. Eventually these invading tissues reached the defect and spread through it into the medulla.

Experiments similar to the latter, except that wax was injected into the marrow cavity at each end to prevent revascularization by metaphyseal vessels, gave essentially similar results, the marrow being revascularized by vessels growing from the gluteal ridge.

The results indicated that there were no effective anastomoses between the periosteal, nutrient and metaphyseal vessels. The cortex and marrow, except for the metaphyses, were supplied from the nutrient artery alone.

Histological changes occurring in the long bones of chickens dwarfed by prolonged undernutrition. By C. W. M. PRATT and R. A. McCANCE. *University of Cambridge*

Rhode Island Red cockerels, which normally weigh over 3000 g. by 26 weeks, were allowed to grow normally for the first 14 days after hatching. They were then held at a body weight which did not exceed 130 g. for varying periods up to 26 weeks. The long bones continued to grow very slowly in length. The cells of the diaphysial cartilage continued to hypertrophy with concomitant exhaustion of the proliferative zone. The endochondral bone formation that followed resulted in a large metaphysis containing slender trabeculae. Any periosteal bone which was formed was compact and showed cement lines. Some of the diaphysial bone adjacent to the medullary cavity was absorbed. There was, however, no absorption of the bone which surrounded the Haversian spaces in the part of the diaphysis which persisted. There was considerable absorption of the secondary spongiosa of the metaphysis in the early stages. After 26 weeks of undernutrition the marrow in the humerus had been completely replaced by an air sac, although this process had not commenced in a control bone of the same length.

Mechanism of ova-implantation in the rat. By M. C. SHELESNYAK. *Weizmann Institute of Science, Rehovoth, Israel*

Evidence is presented demonstrating the existence of functional, pharmacological, and perhaps even physiological, relationships between the ergocornine-ergocristine-ergokryptine complex and progesterone metabolism, although the mechanism of action has not been elucidated. The roles of estrogens and progestogens in establishing the progravid uterus are well confirmed, as is the need for progesterone by the active decidual cell. The relationship of sex steroids to histamine has been demonstrated. We postulate the release of histamine (somehow) by the blastocyst and that histamine (DIF?) plus progesterone operating on a progravid uterus are required for successful decidua development. The

postulated sites of blockage by the antihistamines, epinephrine, and the ergotoxine complex is indicated; complete proof is lacking. We feel that the information which is rapidly accumulating with regard to the relationship of mast cells to the reproductive tract status on one hand, and histamine release on the other, and the increasing data on histaminase-sex steroid relationships tend to fortify our visualization of the mechanism of decidua development.

The volumes of the lungs and air sacs in *Gallus domesticus*.

By A. S. KING and D. C. PAYNE. *University of Bristol*

Estimates of the capacities of the lungs and air sacs of birds are rare. Only two are available for *G. domesticus*, by Campana (1875) from one hen and by Zeuthen (1943) from three hens. Both used casts, but Zeuthen failed to fill the sacs.

Ten adult hens of mixed breeds were injected through the trachea and through each humerus with latex, by gravity from about 2 ft. Techniques were used to release air trapped at the ends of the sacs. The volumes of the casts were measured with a density bottle. The mean volume in ml. with standard deviations were: left lung 9.3 ± 2.1 , right 8.8 ± 2.4 ; cervical sac 8.3 ± 2.8 ; interclavicular sac 27.1 ± 3.7 ; left cranial thoracic sac 15.4 ± 3.8 , right 14.6 ± 4.9 ; left caudal thoracic sac 4.2 ± 1.2 , right 5.4 ± 2.0 ; left abdominal sac 95.9 ± 16.1 , right 112.1 ± 48.9 .

The latex shrank by about 25 %. After correction for this a rough estimate of the maximum capacities of each lung, the interclavicular sac, each cranial thoracic sac, and each caudal thoracic sac, would be 12, 35, 20, and 6 ml. respectively. The corrected estimates for the cervical sac (about 11 ml.) and abdominal sacs (about 130 ml. each) are too low through incomplete filling.

The bronchial tree in aquatic mammals. By D. BROWN.

Charing Cross Hospital Medical School, London

Marco resin casts have been prepared in order to illustrate the principal gross features of the lungs and bronchi of the otter, seal, sea-lion and porpoise.

All of these animals except the sea-lion show a degree of gross pulmonary symmetry and the seal shows also complete bilateral symmetry of its bronchial tree. The porpoise lungs on the other hand show a distinctly unequal bronchial pattern, including a tracheal bronchus on the right side. In addition, presumably as an adaptation for diving, the centre of each lung has become filled with connective tissue so that the respiratory area is condensed at the periphery, appearing in the cast like 'pie-crust' outside the bronchial tree.

The sea-lion lungs, retaining the typical terrestrial carnivore pattern, are an enigma which so far defies satisfactory explanation.

Musculus palmaris longus in aquatic mammals. By K. M. BACKHOUSE.

Charing Cross Hospital Medical School, London

Musculus palmaris longus is often considered to be somewhat atavistic in the mammalian series but it is of considerable importance in certain mammals highly specialized for aquatic life.

In all the Pinnipedia, *m. palmaris longus* is a complex muscle, one part of which runs along the postaxial border of the limb, being essentially a postaxial deviator of the manus, an abductor digiti quinti longus.

The Sirenia have no flexor component of *m. palmaris longus*, the whole muscle running down the postaxial border of the limb, to gain insertion into the postaxial border of the manus and digitus quintus.

The Cetacea have a small number of relatively rudimentary ante-brachial muscles

but one of these runs down the postaxial border of the limb to gain insertion into the pisiform bone (if present), and into the postaxial border of the manus. This muscle, m. flexor carpi ulnaris, has close functional affinities with the m. palmaris longus of the Pinnipedia and the combined mm. palmaris longus and flexor carpi ulnaris of the Sirenia.

The functional significance of these adaptations was considered.

The cetacean ear. I. The osteology of the skull base.

By F. C. FRASER. *British Museum (Natural History), London*

The cetacean ear is comparable with that of terrestrial mammals, having representatives of all the essential structures of the latter. It receives water-borne vibrations differing in pressure and displacement amplitudes from those which are air-borne. Vibrations presented at the fenestra ovalis require to be those normal in mammalian hearing. Modifications of the components of the middle ear ensure the conversion of the water-borne vibrations. The proper functioning of the mammalian cochlea requires molar movement of the auditory ossicles. This in turn demands an air space at all conditions of external pressure, as does also the membrane of the fenestra rotunda. Hydrostatic balance on either side of the tympanic membrane is also concerned. The cavity of the middle ear is extended in all cetaceans into an air sinus system which involves the pterygoid bone. The latter is extended, invaded, inflated, and has parts of its bony content removed. Variations in the extent of these processes occur in the cetaceans as a whole. The external lamina of the pterygoid may be complete (*Platanista*), fenestrated (*Lissodelphis*) or altogether removed (*Inia*). Residual portions of the bony content of the external lamina may persist (*Phocaena*).

The extension of the still bony sinus-enveloping pterygoid into the orbital regions is demonstrated in *Pseudorca*. In *Delphinus* the grooving of the palate almost to the tip is an expression of the sinus extension. Not only the pterygoid itself but the bones adjacent to it are profoundly modified by the sinus system. The architecture of the skull bones is made intelligible by taking the sinus system into account.

The cetacean ear. II. The middle ear with special reference to the soft parts of the air sinuses. By P. E. PURVES. *British Museum (Natural History), London*

When the basicranial region of the cetacean is dissected to expose the air sinus system a number of intricate blood systems are found for which no homologues can at first sight be established in the vascular system of terrestrial mammals. Injection of coloured polyester resins into the air sinuses and into the vascular system makes possible the identification of the homologues of these apparently new blood systems.

The internal cast of the air sinus shows that it occupies the interlaminar region of the pterygoid bone and the space between the tympano-periotic bones and the skull. The sinus is also continuous with the tympanic cavity and the Eustachian tube and can be regarded as a diverticulum of the latter.

As a consequence of the invasion of the pterygoid lamina of the peribullary region by this diverticulum, there has occurred during evolution extensive removal of the calcified matrix of the bone, leaving behind Haversian blood systems which have subsequently hypertrophied. The removal of bone has also resulted in complete dissociation of the tympano-periotic bones from the cranium. In the recent cetacea the whole air sinus system is filled with a stable foam formed from an oil-mucus emulsion. This foam is a very good sound insulator, reflecting over 99 % of the energy of all sound waves impinging on the head, except those in the region of the external auditory meatus. This factor endows the sense of hearing in cetacea with directionality. There is evidence that with increasing hydrostatic pressure, contraction of the air space is compensated for by injection of blood into the vascular systems described above.

Vascular modifications and experimental diving in *Phoca vitulina*.
 By R. J. HARRISON and J. D. W. TOMLINSON. *London Hospital Medical College*

In a previous communication (*Proc. zool. Soc. Lond.* 1956, **126**, 205) we described certain modifications in the venous system of Pinnipedia that may be associated with diving bradycardia. A number of pups and 2-year-old adolescents of *Phoca* were dived experimentally in shallow tanks at sea and in a pressure chamber for periods up to 25 min. and to depths up to 300 ft. Recordings were made on a portable Elmquist electrocardiogram. The results were compared with those recorded by Scholander (*Hvalrad. Skrift.* 1940, **22**, 1) on pups of *Halichoerus* and *Cystophora*. They showed that in *Phoca* the heart rate slowed from a variable resting rate of 55–180 to a minimum recorded rate of 4 beats per min. between 1–3 min. after diving. Thereafter the rate gradually increased as the dive continued. Animals easily withstood diving to depths of 80 ft.; one animal survived apparently unharmed for over 1 min. at 300 ft., but succumbed on surfacing after 5 min. at that depth. Struggling developed if the animal was prevented from exhaling under water. Increased air pressure equivalent to depths of 33, 100 and 200 ft. did not produce marked bradycardia, but irregular slowing correlated with respiratory rate. Anaesthesia of the large infra-orbital nerves did not influence bradycardia, section of one vagus caused irregular slowing, section of both vagi was not followed by bradycardia on diving.

Experiments on the phrenic nerve in *Phoca vitulina*. By J. D. W. TOMLINSON,
 R. J. HARRISON and R. S. WARWICK. *The London Hospital Medical College and Guy's
 Hospital Medical School, London*

The right phrenic nerve in *Phoca* is of particular interest in that it supplies a well-developed caval sphincter of striated muscle lying cranial to the diaphragm. It arises mainly from C₅ and communicates in the neck with the sympathetic chain and with a ganglion lying close to and having connexions with the recurrent laryngeal nerve. The left phrenic nerve does not supply the sphincter, and has no demonstrable connexions with either the sympathetic chain or recurrent laryngeal nerve nor is there a ganglion associated with the latter. Fibre counts have been made on the two sides, at varying sites, of the phrenic, recurrent laryngeal, vagus and sympathetic trunk.

Stimulation of the right phrenic nerve exposed in the thorax causes the sphincter to contract and occlude the caval venous return, which can be demonstrated both visually and by injection of radio-opaque material. Neither such occlusion nor prolonged manual compression has any appreciable effect on the heart rate. Section of the right phrenic nerve in the neck and section of its branches to the sphincter have been performed, the animals allowed to recover, and then dived in a shallow tank to determine the effects of phrenic section on diving performance. Animals were killed 10 and 12 days after section of the right phrenic in the neck and the spinal cord examined for degenerative changes to determine the presence or absence of a phrenic nucleus.

Organization and development of the preterminal nervous pattern in the palmar digital skin. By N. CAUNA and G. MANNAN. *King's College, Newcastle upon Tyne*

Fingers of 200 individuals ranging from 16 mm crown-rump length to 93 years of age have been studied in frozen and serial paraffin sections stained for nerves.

It has been found that digital nerves consist of single medullated axons and bundles of fine non-medullated fibres. While approaching the skin these fibres are gradually segregated according to their type and destination. Articular and periosteal fibres are given off first, followed by thick single medullated axons supplying Pacinian corpuscles. Next, all non-medullated autonomic fibres accompanied by some fine medullated axons leave the corial nerves to form an independent subcorial and perivascular plexus which supplies blood vessels, arterio-venous anastomoses and sweat glands; this plexus becomes exhausted on

reaching the papillary layer. The remaining medullated fibres of the corial nerves form the deep and superficial corial plexuses which supply the receptors of the skin.

The development of the corial plexus coincides with that of the papillary ridges and sweat glands. Thick fascicles of nerve fibres which have almost reached the epidermis at 30–60 mm. stages, give origin to horizontal branches which grow into the developing corium. As the latter increases in thickness, the thick fascicles retreat from the epidermis to their permanent situation.

Extension of a freckle associated with stretching of a scar.

By A. S. BREATHNACH. *St Mary's Hospital Medical School, London*

An originally linear scar on the forearm of a freckled individual became stretched over a period to a width of about 7 mm. Associated with this, a small freckle lying along one edge of the scar was observed to have increased in size four or five times, so as to occupy an area of 12 mm.² of the new epidermis over the stretched scar.

Examination of Dopa-incubated 'split-skin' preparations of pigmented and pale epidermal areas of the scar revealed melanocyte patterns identical with those of corresponding parent epidermal areas along the edge. Clear-cut differences in morphology, distribution, and Dopa-reactivity existing between melanocytes of pale and pigmented (freckle) parent epidermis were reproduced over the scar. How far this can be taken as evidence that melanocytes of freckled subjects breed true in respect of these differences was discussed.

Light- and electron-microscopic observations on postnatal stages of the rat submaxillary gland.

By C. R. LEESON and F. JACOBY. *University College, Cardiff*

The adult glandular lobule contains four main epithelial elements in the following spatial order: (1) the acini; (2) intercalated ducts; (3) convoluted so-called 'special serous' segments; (4) secretory ducts (*Streifenstücke*). Alk. phosphatase-positive myoepithelial cells are present around (1) and (2). E.M. reveals as the most striking features a complex system of endoplasmic reticulum, sparse mitochondria and nuclei of great density in (1); numerous deep infoldings of the cell membranes in (4) and shorter ones in (3); apical secretory granules of varying density and size in (3) and numerous basal mitochondria in (3) and (4).

Studies of glands of rats of known ages from 2 weeks to 6 months show, both by light and E.M., that the distal portions of the secretory ducts are well differentiated very early; their proximal portions, the differentiation of which into 'Streifenstücke' follows, become, from 6 weeks on, gradually transformed into the convoluted 'special serous' segments. The basophil cells of the definitive acini arise as buds from acidophil granular precursors which stain most intensely at 4 weeks. The greater electron density of the acinar-cell nuclei is present right from the start, but the endoplasmic reticulum is much less in evidence.

The effect of hypoxia upon bone marrow volume.

By G. HUDSON. *University of Bristol*

For quantitative studies of the response of erythropoietic bone marrow to hypoxia, it is essential to know whether changes in its total volume have occurred.

Ten guinea-pigs of approximately 400 g. body weight were kept for 14 days at a simulated altitude of 20,000 ft., a comparable group being kept for a similar period under control conditions. Each animal was then killed and the extent of red and fatty marrow noted in limb bones of one side. The rest of the skeleton was used for estimation of bone marrow volume by a modified agar method previously described (*J. Anat., Lond.*, 1958, 92, 150.).

After 14 days' hypoxia, fatty marrow was still present in the usual sites at the periphery of the limbs.

The mean total bone marrow volume in controls was 7.382 ml. (standard deviation 0.446 ml.) of which 6.594 (0.418) ml. was red marrow. The corresponding figures after 14 days' hypoxia were 7.420 (0.421) ml. and 6.625 (0.387) ml., i.e. no increase in bone marrow volume was found beyond that occurring in normal growth.

Macerated skeleton weights were significantly less in the hypoxic animals.

The influence of 4-methyl-2-thiouracil on the teratogenic activity of hypervitaminosis-A. By D. H. M. WOOLAM and J. W. MILLEN. *University of Cambridge*

In previous communications to the Society we described the action of cortisone in augmenting the teratogenic activity of hypervitaminosis-A and the abolition of this effect by insulin. The present communication has recorded the effects of another agent, 4-methyl-2-thiouracil, when administered in conjunction with vitamin A.

Three groups of female rats were used in these experiments. Animals in groups 1 and 2 received 60,000 i.u. of vitamin A by gastric intubation daily from the 8th to 13th days of pregnancy inclusive. Those in groups 2 and 3 received a 0.1% solution of 4-methyl-2-thiouracil in lieu of drinking water, from the 1st to 10th days of pregnancy inclusive.

The rats were killed on the 20th day of pregnancy; the young removed and examined for deformities. No malformations were found in the young from group 3. Of the young from Group 2, 68.8% showed deformities of the brain and calvaria as compared with 7.8% of the young from group 1. The findings indicated that 4-methyl-2-thiouracil augmented the teratogenic activity of hypervitaminosis-A.

The morphology of the autonomic ground plexus in the gut wall.
By J. R. RINTOUL. *University of Manchester*

The autonomic ground plexus as seen in the myenteric plexuses, examined by different methods, presents a microscopic appearance which varies as the methods of fixation and staining are altered. In consequence, diverse interpretations of these appearances have been made. Employing a modified Bielschowsky technique, the ground plexus is seen to consist of a system of fine nerve fibres arranged in bundles. Using the method of Jabonero (*Acta Anat.* 11, 1950-51), the ground plexus assumed the appearance of a terminal syncytium of nucleated protoplasm, with no nerve fibre differentiation: he considers 'the interstitial cells of Cajal' form an integral part of the system of anastomosing bands of neuroplasma. Those using electron microscopy techniques suggest that the interstitial cells resemble fibroblasts.

These conflicting findings are due in part to the application of the term 'interstitial cell' to different elements. Evidence for and against the various theories regarding the nature and function of these cells are adduced from preparations of material from different vertebrate animals, and specimens of colon removed for the treatment of Hirschsprung's disease.

The origin of the acoustic ganglion in the sheep
By E. H. BATTEN. *University of Bristol*

Both the VII and the VIII ganglia are generally regarded as arising from a common 'acoustico-facial neural crest'. In the sheep, however, this primordium is correctly identified as the facial crest since it is exclusively concerned in the formation of the geniculate ganglion after receiving a contribution from the facial epibranchial placode. The early rudiment of the acoustic ganglion is independent of the facial crest and appears at the 6 mm. stage as a small mass of placodal cells at the base of the otic vesicle. With the addition of further placodal cells which detach as spurs and files from the otic epithelium the enlarging acoustic ganglion fuses with the geniculate ganglion by the 8 mm. stage, but the latter can be distinguished by the advanced differentiation of its neuroblasts.

The definitive vestibular ganglion develops from the pars superior together with the lateral zone of the pars inferior from which the inferior vestibular nerve extends. The placodal cells of the medial zone migrate ventrally and, augmented by diffuse placodal contributions during the 11–14 mm. stages, mass into the cochlear lobe, which later enters the axis of the spiral cochlear duct to establish the definitive cochlear ganglion by the 26 mm. stage.

Boiled foreign cartilage grafts in relation to transplantation immunity.

By M. B. L. CRAIGMYLE. *University College, Cardiff*

In a previous communication it was shown that fresh foreign cartilage grafts appeared to be antigenic, evoking regional lymph node changes identical with those elicited by foreign skin grafts. Billingham *et al.* (*Nature, Lond.*, 1956, **178**, 514) reported that cell suspensions, normally highly active in eliciting transplantation immunity, lose their antigenicity after heating to 48·5° C. From this, the question arose whether grafts of boiled foreign cartilage, such as used in plastic surgery, were antigenic. Accordingly, the regional lymph node changes induced in the rabbit by fresh and boiled bovine cartilage were compared.

Nine (control) rabbits bore cartilage autografts, nine fresh bovine and nine boiled bovine cartilage heterografts in one ear for four days, after which time the regional lymph nodes of both sides were secured. No weight or cytological change occurred in nodes from control rabbits. A marked (190–400 %) weight increase of the node taken from the operated side was found in rabbits bearing fresh bovine cartilage, and the cortex of the node contained many pyroninophilic large lymphoid cells. The weight increase in rabbits which had had boiled bovine cartilage was much less (37–133 %) and fewer large lymphoid cells were seen. The significance of these findings was discussed.

The fate of callus transplants in the rat and rabbit.

By W. THOMPSON. *The Queen's University, Belfast*

The fate of fibro-cartilaginous fracture callus transplanted to the brain was studied in rats and rabbits, and of similar transplants placed under the kidney capsule in rats.

Fresh autogenous and homogeneous transplants in the brain behaved similarly. They were eroded, vascularized, and replaced by bone in both rats and rabbits, but these changes were much more rapid in the rat. In the latter animal the transplants were converted within 3 weeks into an approximately spherical ossicle, filled with haemopoietic marrow. Fresh autogenous transplants under the kidney capsule in the rat underwent similar changes, except that the ossicle which eventually formed was lens-shaped. Fresh heterogeneous (mouse) callus transplanted into the rat's brain was also eroded and vascularized, but much more slowly than autogenous or homogeneous transplants. In one instance bone was formed. In the rabbit, homogeneous transplants, devitalized with absolute alcohol and placed in the brain were fairly rapidly eroded and new bone was induced as early as 30 days after transplantation. In the rat erosion was much slower and bone was not seen until 100 days after transplantation.

These experiments illustrated the remarkable capacity for self-differentiation and re-organization possessed by fibro-cartilaginous fracture callus. They indicated, moreover, that some of these effects may be mediated by chemical inductors. The environment, however, appeared to be responsible for determining the external form of the ossicle which was eventually produced.

'Assimilatory induction' in cartilage.

By J. P. BALMER. *The Queen's University, Belfast*

According to Lacroix (*Bull. Acad. roy. Med. Belg.* 1945, **10**, 512) non-ossifiable hyaline cartilage from the rabbit will become hypertrophic and support endochondral ossification when placed in contact with epiphyseal growth cartilage. He termed this phenomenon

'assimilatory induction'. In the present study non-ossifiable costal cartilage was placed in a hole drilled through the upper epiphyseal plate of the tibia in rabbits, rats and guinea-pigs, and also in apposition with epiphyseal cartilage under the kidney capsule in rabbits and rats.

Cell column formation, cell hypertrophy and endochondral ossification occurred in autogenous transplants of costal cartilage to the tibia in the rabbit and guinea-pig. In the rat and guinea-pig, however, similar homogeneous transplants were eroded, vascularized, and showed endochondral ossification but hypertrophic changes in the cartilage were equivocal. Costal cartilage in apposition with epiphyseal cartilage under the kidney capsule of the rabbit showed cell column formation, but no hypertrophy or bone formation. No such changes were observed in the rat.

It was concluded that in certain circumstances hyaline cartilage may become vascularized and replaced by bone in the rat, rabbit and guinea-pig. In the latter two animals hypertrophic changes may occur in the cartilage preparatory to ossification. Proximity to epiphyseal cartilage seemed only to induce the formation of cell columns preparatory to hypertrophic changes and endochondral ossification in the rabbit, whereas proximity to bone marrow appeared to induce full maturation of the cartilage and endochondral ossification in both rabbit and guinea-pig.

Sites of calcium deposition in experimental nephrocalcinosis produced by injections of calcium or of phosphate. By JULIA M. FOURMAN. *University College, Cardiff*

Rats injected intraperitoneally with 13 mg. of calcium as the gluconate daily for 10–24 days deposited calcium in their kidneys. The calcium content of these kidneys varied from 3 to 9 mg. (1 mg. normal) per g. of fat-free dry kidney. Histochemically the calcium was found only in the basement membrane of the first part of the proximal convoluted tubules. Rats injected on alternate days, 8–15 times, with a neutral sodium phosphate solution containing 60 mg. of P per dose deposited similar amounts of calcium in their kidneys, but this was histologically seen to be much more widely distributed. It was mainly found in the basement membrane of the tubules of the medullary rays. The parts of the nephrons chiefly affected were the terminal straight parts of the proximal convoluted segments, and more distal portions, either the thin descending limbs of Henle's loop or the small collecting tubules.

'Fragilitas ossium' in a Patas monkey. By W. R. M. MORTON.
The Queen's University, Belfast

Multiple fractures of the long bones of an 18–24 months' old male Patas monkey were found to be associated with almost complete destruction of the cortical bone, which was replaced by an excess of fibrous tissue. The bones were so demineralized that they could be cut across with a knife. On microscopic examination they showed a thin ring of ossified material centrally from which trabeculae radiated centripetally. These trabeculae were ossified at their bases but peripherally were composed of calcified cartilage. Fibrous tissue capped the trabeculae and interdigitated with them. Masses of cartilage were present in the excess callus around the fracture sites. The microscopical appearances closely resemble those caused by vitamin-C deficiency as described by Murray & Kodicek (*J. Anat., Lond.*, 1949, 83, 205). The parathyroid glands were not located, but the thyroid showed evidence of over-activity.

Postnatal obliterative changes in the rabbit ductus arteriosus.
By R. L. HOLMES. *University of Leeds*

The ductus arteriosus was studied histologically and histochemically up to the 6th post-natal week. Following contraction, there appeared to be an avascular necrosis of the inner media. Histochemical studies showed the absence of such enzymes as alkaline phos-

phatase, esterase, succinic dehydrogenase, peroxidase and β -glucuronidase in this zone, but some activity was present in cells surrounding the residual lumen and in the normal muscle of the outer media. Colchicine-treated animals showed early mitotic activity in the intimal region.

Alcohol-resistant metachromasia was very slight in the inner medial tissue, but some young specimens showed a band of pronounced metachromasia at the junction between the inner media and the surrounding muscle. No actual increase in amount of elastic tissue was noted during the obliterative changes. High dosage of cortisone acetate over the first ten days of life did not prevent the histological occlusion.

It was considered that the obliterative process consisted of an early inner medial necrosis, with initial survival and slight proliferation of cells immediately surrounding the residual lumen; and that this was followed by gradual organization of the dead tissue accompanying revascularization.

A regulatory mechanism in the uterine artery of the rat.

By D. B. MOFFAT. *University College, Cardiff*

The uterine artery in the rat runs parallel with, and a short distance from the corresponding horn of the uterus and gives off branches to the horn which leave the main trunk at approximately right angles. At the origin of each of these branches is found a pair of valve-like lips which project into the main vessel and which are partly composed of longitudinally running smooth muscle fibres. These are embedded in a variable amount of matrix which stains metachromatically with azore A. The metachromatic staining is particularly intense before puberty. It is suggested that these structures regulate the amount of blood passing to the uterus and may be responsible for the zones of constriction which have been reported in the branches of the uterine artery by a number of workers.

A diaphragmatic hernia in an adult cat. By R. L. HOLMES

and W. K. J. WALLS. *University of Leeds*

An extensive literature on diaphragmatic hernia in man exists but reports of such herniae in animals are relatively few. A case of gross hernia in a 7-year-old apparently healthy cat is described. Part of the stomach, all the small intestine, part of the large intestine, together with the spleen, pancreas and part of the liver are found in the right pleural cavity. The hiatus lies in the dorsal part of the right half of the diaphragm and there is no hernial sac. The defect is thought to be congenital.

JUNE 1958

The Summer Meeting of the Society for the Session 1957-8 was held on Friday and Saturday, 27 and 28 June 1958, in the Anatomy Department, University of Aberdeen. The President (Prof. F. GOLDBY) and the Vice-Presidents (Prof. R. WARWICK and Prof. J. PRITCHARD) occupied the Chair at the various sessions.

The following are the abstracts of the papers read:

The embryology of first arch abnormalities. By J. MCKENZIE,

University of Aberdeen

The numerous congenital abnormalities of the first visceral arch in man are usually regarded as separate entities without reference to any common basic aetiological factor. A study of their hereditary features, however, suggests that they may be closely linked.

Dissection of a case described previously indicates that the blood supply to the first arch during the third to the fifth week may play a significant part in the production of

these anomalies and it can be shown from the normal embryology of this region how one derangement in its vascular development may lead to one or several of the clinical conditions. The entire range of first arch abnormalities should therefore be considered as comprising one syndrome.

Alterations in the vascular pattern in chick embryos following the injection of insulin. By J. MCKENZIE and D. E. B. CHAYTOR. *University of Aberdeen*

The relative frequency of congenital anomalies of the first visceral arch occurring naturally in man and other animals, as well as the vulnerability of the region in experimental teratogenesis, prompted a comparison of its normal development with that of the abnormal.

Eggs were injected with insulin at 4–4½ days' incubation and the embryos examined at 6, 6½ and 7 days, when, in addition to the general retardation in growth, there was already gross maldevelopment of the beak. The blood supply to this region was examined in serial sections and reconstructions made. Retardation in growth of the definitive vessels to the first arch was evident in their poorly developed walls and in the presence of compensatory anastomoses with other arteries. The significance of these findings was discussed.

Self-regulation in insulin-injected embryos. By D. E. B. CHAYTOR.
University of Aberdeen

Eggs have been injected with insulin at 4–4½ days of incubation. The beaks and legs have been measured in a series of embryos aged 8–19 days. The results indicate that embryos can recover from the injurious effects (e.g. short upper beak, parrot beak) induced by insulin. The ability to recover varies with the individual embryo and is evident from 12 days onwards. When growth rates are compared in normal and abnormal series, deviation is observed in the pattern of the abnormal during the period of self-regulation.

These findings have been explained on the basis of the presence of modifying genes in the stock used (Light Sussex × Brown Leghorn). Landauer (1946, *Amer. Nat.* **80**, 490) has found that, in the hereditary condition of short upper beak, modifying genes may so act that the beak sometimes becomes larger than normal.

An abnormality of the pancreatic ducts associated with duodenal and jejunal diverticula. By A. DEAN. *University of Aberdeen*

The specimen was obtained from a dissecting room subject aged 67 who died of congestive cardiac failure. The main pancreatic duct passes across the front of the duodenum to enter the bowel on its posteromedial aspect, separate from the common bile duct. The abnormality may possibly be due to the persistence of the left ventral pancreatic bud and its relation to the condition of annular pancreas is discussed. There are four diverticula of the duodenum and one of the jejunum. An accessory pancreatic duct opens on a septum in one of the diverticula. The surgical importance of the findings is emphasized.

A quadruplet placenta. By W. J. HAMILTON, B. G. SPIERS and D. BROWN. *Charing Cross Hospital Medical School and East End Maternity Hospital, London*

In December 1957, full-term living quadruplets were delivered by Caesarian operation at the East End Maternity Hospital, London; the infants, two boys and two girls, weighed

	lb.	oz.		lb.	oz.
Boy	5	7	Girl	4	1
Boy	5	1	Girl	3	14

The placental mass was obtained in the fresh state and each umbilical vein and one of each pair of umbilical arteries were injected with coloured gelatine. There was no macroscopical evidence that the circulation of one placenta was continuous with that of its neighbour and sections across the lines of fusion of the placentae substantiated this when examined microscopically. The findings were discussed in the light of subsequent knowledge of the individual infants.

Periodic acid-Schiff positive materials in the rat placenta. By A. D. DICKSON and D. BULMER. *University of Aberdeen*

Rat placentae were investigated by the periodic acid-Schiff technique. Glycogen, identified by digestion with malt diastase, was found to be situated mainly in the junctional zone of the trophoblast and the decidua, small quantities occurring in the labyrinthine syncytium and, possibly, the giant cells. Many structures, including Reichert's membrane, the uterine epithelium, the intercellular material of the decidua, some of the granules of the metrial gland cells, the distal portions of the cells of the visceral layer of the yolk-sac and some cytoplasmic contents of the giant cells, showed diastase-fast PAS reactions. Attempts were made to identify some of the materials responsible.

Early implantation in the lesser bush baby (*Galago senegalensis*).
By H. BUTLER. *University of Khartoum*

The centrally implanted blastocyst, some 4·5 mm. in diameter, is bilaminar, having an outer trophoblastic shell surrounding a capacious yolk sac; no mesoderm is present. An embryonic disc and a large pro-amnion are situated at its mesometrial pole. For the most part, the trophoblast is in close apposition with the intact uterine epithelium but cytoplasmic processes intrude between uterine epithelial cells. Over the mouth of each uterine gland the thickened trophoblast, the pro-chorionic vesicles, is actively absorbing glandular secretion. Over an area (some 2·5 by 4·0 mm.) of its mesometrial pole the trophoblast forms a single layer of giant cells which are firmly attached to the maternal connective tissue. The endothelial-like endoderm cells of the avascular yolk sac are closely applied to the inner surface of the giant cells. Maternal capillaries are in close contact with the outer aspect of the giant cells. For a distance of 200 μ around the periphery of the giant cell trophoblast the uterine epithelium is undergoing syncytial degeneration. Products of degeneration are being absorbed by the trophoblast and adjacent part of the yolk sac, whose cells are here swollen and vacuolated.

Observations on the origin of the prostatic utricle in Man. By T. W. GLENISTER.
Charing Cross Hospital Medical School, London

The development of the utricle has been traced up to the eighth month of pregnancy and four phases of development have been noted. The first phase comprises the stages when the caudal fused ends of the paramesonephric ducts are joined to the urogenital sinus by a solid 'vaginal' cord. In the second phase this cord lengthens and is separated from the sinus by bilateral extensions of sinus epithelium that fuse in the midline and give rise to a single sinu-utricle cord.

In the third phase the paramesonephric remnant is confined to the developing prostate and consists of a solid cord of cells merging imperceptibly with the sinu-utricle cord. In subsequent stages the composite rudiment acquires a lumen and dilates enormously. This is associated with stratified squamous hyperplasia and the outgrowth of cellular buds into the surrounding stroma, the epithelial changes being more marked near the urethra.

It has been noted that the epithelium lining the urogenital sinus and, later, the urethra in the region where they are joined by the mesonephric and paramesonephric ducts (or sinu-utricle cord), presents special histological characteristics. The subsequent fate of

the utricle, its glands and those arising from the sinus epithelium near the utricular orifice, is being studied with reference to their possible role in middle lobe hyperplasia of the prostate.

The epithelium of the urogenital sinus and vestibule in female human foetuses.
By D. BULMER. *University of Aberdeen*

The epithelium of the urogenital sinus has been studied in a series of female foetuses, and in a one-month infant. Up to the 140 mm. stage, excepting where it gives rise to the vaginal primordium, the sinus is lined by a darkly staining stratified epithelium, and this is clearly distinguished from the epithelium of the developing vagina above and from the epidermis over the inner aspects of the urethral folds below. After the 140 mm. stage, the darkly staining sinus epithelium is largely replaced by an epithelium which is histologically and histochemically similar to the vaginal epithelium, but remains distinct from the keratinized epithelium over the labia minora. In addition, however, the developing vestibule contains patches of another epithelium, often of stratified columnar form and containing alveolar spaces lined by cells which give a diastase-fast PAS reaction. These are, presumably, the precursors of the lesser vestibular glands. In the one-month infant the distinguishing features of the different epithelia are less marked, suggesting their dependence, in part, on hormonal stimulation.

The early development of the nasal cavity and upper lip in the human embryo.
By J. G. WARBRICK and R. J. SCOTHORNE. *University of Glasgow*

The early development of the nasal cavity and upper lip has been investigated in a series of human embryos ranging from 7·5 to 16 mm. Study of sections and of wax plate reconstructions provides no convincing evidence to support the widely held view that the maxillary process 'overgrows' the fronto-nasal process and provides the mesodermal basis of the central part of the upper lip and alveolus. Streeter's description of the development of the primitive posterior naris as the result of the breakdown of an epithelial 'nasal fin' is confirmed. The embryological evidence for the separation of the epithelium of the roof of the mouth from the subjacent (paraxial) mesoderm by a medial spread of maxillary mesoderm ('septal process') is called in question. The validity of the concept that adult innervation patterns necessarily provide trustworthy evidence of developmental mesodermal migrations was discussed in relation to actual conditions in the embryo.

An index for a hundred years of the *The Journal of Anatomy*.
By R. E. M. BOWDEN. *Royal Free Hospital Medical School, London*

The first issue of the *Journal of Anatomy and Physiology* was published in 1867. An Index for the first twenty volumes appeared in 1894 and another for the next ten volumes was published in 1897; both these and subsequent indices are now out of print. The Council has decided that it would be of great value to have a full Index covering the first hundred years of the Journal and the Proceedings of the Society. The Librarian of the Royal Free Hospital, Mrs D. Blake, has undertaken its compilation and the Cambridge University Press is publishing the Index for the Society.

Construction of such an Index entails numerous decisions on problems of cross-reference and typography. These problems were discussed, samples of layout and type shown and a progress report was presented.

Mobility of the arch of the foot. By R. D. LOCKHART. *University of Aberdeen*

X-rays and photographs were shown to demonstrate the range between the highest and lowest state of the arch, and further, that the arch rises equally whether one is standing on the toes or standing on the foot with the toes dorsiflexed.

Loading in the upright position of the body does not depress the arch.

Although these points are not new there is controversy and the writer's views have been misapplied.

While it is stated that in the standing posture concentric needle electrodes show activity only in the posterior leg muscles, the point is made that the anterior leg muscles at least feel firm even in forward inclination of the trunk.

The effect of temperature on the maturation of regenerating peripheral nerves.

By F. GOLDBY and B. D. JHA. *St Mary's Hospital Medical School, London*

Previous investigations on the caudal nerves of the rat showed that both degeneration and the early stages of regeneration were accelerated by warmth and retarded by cold (Gamble & Jha, 1958; Gamble, 1958). This effect was further investigated in the rat's sural nerve, the animals being maintained in environmental temperatures of about 37°, 21° and 5° C. for periods up to 150 days. The subcutaneous temperatures close to the regenerating nerves were about 37°, 35° and 32° C. respectively.

It was found (by counting in transverse sections) that up to about 70 days after operation the total number of myelinated fibres in the regenerating nerve was smaller in the 'warm' than in the 'hot' animals, and smaller again in the cold. The effect was most marked in the 50-day specimens. After 70 days the total numbers of fibres in the 'hot' and 'warm' animals did not differ significantly and approximated to the number in the normal nerve, though in the 'cold' animals they remained slightly below this level throughout the period of the experiment. Histograms showed that maturation (judged by the proportions of coarser fibres) was less advanced in the cooler than in the warmer animals at all periods studied.

The quantitative data indicated that differences of the order of 2-3° C. in the temperature of the regenerating nerve (within the range of 30-37° C.) may lead to a retardation of maturation by about 20-30 days within the period from about 50-150 days after injury, but the general effects of exposure to abnormal environmental temperatures may be a complicating factor in experiments of this kind.

An effect of pyronin upon the maturation of peripheral nerve fibres.

By H. J. GAMBLE and B. D. JHA. *St Mary's Hospital Medical School, London*

Hoffman (*Aust. J. exp. Biol. med. Sci.*, **30**, 1953) has reported that the outgrowth of buds from normal axons into partially denervated muscle was accelerated by feeding 0·1% pyronin to rats. His investigation had been prompted by the claim that pyronin is mutagenic in *Drosophila* and accelerates the rate of growth, possibly by attaching itself to and by action upon nucleic acid bodies.

The sural nerve was crushed in fourteen young adult rats and allowed to regenerate. Six of these animals were given 0·1% pyronin to drink and were killed in couples after 30, 50 and 70 days. The eight other animals drank tap water and were killed after similar intervals. Transverse sections were treated by the Guttman & Sanders technique. The myelinated fibres present were counted and measured.

The results suggest that, after 30 days, nerves from the pyronin-fed animals contain more myelinated fibres and, after 50 and 70 days, larger numbers of large-diameter fibres than do the controls. The total cross-sectional area of the myelinated fibres is considerably larger in the nerves from the pyronin-fed animals, and an Analysis of Variance has shown that this difference is highly significant.

Observations on the development of nerves in embryonic rats.

By A. PETERS and A. R. MUIR. *University of Edinburgh*

Structural changes in the peripheral nerves of rat embryos, from the 13th day of gestation to birth, have been studied by light and electron microscopy. The early embryonic nerve consists of a bundle of small, tightly packed, naked axons separated from the surrounding

tissue by a layer of Schwann cells. Later, tongues of Schwann-cell cytoplasm appear within the nerve between bundles of naked axons; these tongues of cytoplasm then separate the naked axons, so that many become enclosed within each Schwann cell. As the Schwann cells divide, the number of axons related to each cell is reduced, so that before myelination each Schwann cell contains but one axon. The changing relationship between Schwann cells and axons is seen in electron micrographs, and the proliferation of Schwann cells is analysed quantitatively by light microscopy.

Connexions of the efferent fibres from the gracile and cuneate nuclei in the macaque monkey. By D. BOWSHER. *University of Liverpool*

The posterior column nuclei were destroyed unilaterally in three macaques. After sacrifice three weeks later the brains were serially sectioned and impregnated by the axon degeneration technique of Nauta.

Efferent fibres from these nuclei were found to be completely crossed, and to terminate exclusively within the contralateral nucleus ventralis postero-lateralis thalami, except for a few fibres ending in the mesencephalic nucleus paralemnisialis. No terminal degeneration, stem or collateral, was seen in the brain stem reticular formation, the ipsilateral thalamus or cerebral commissures, the globus pallidus or any other extrapyramidal motor nuclei.

The significance of these findings was discussed with reference to (i) ipsilateral conduction of afferent impulses, (ii) afferent control of the extrapyramidal motor system, and (iii) the theory of non-specific collateral afferent excitation of the brain stem reticular formation.

The origin and significance of cartilage in bone repair. By J. J. PRITCHARD, J. T. McDANIEL and H. C. MULHOLLAND. *The Queen's University, Belfast*

The origin and significance of cartilage formation in the course of bone repair are still debated. Evidence is presented, based on the histological examination of a variety of repairing bony injuries, in favour of the following hypotheses:

- (1) The cambial cells of the periosteum can form cartilage as well as bone.
- (2) The periosteal cambial cells form cartilage when and where their rate of proliferation is such that new cells accumulate faster than the new blood vessels can supply them adequately for bone formation.
- (3) The rate of cambial cell proliferation depends on age, species, severity of injury, and the intensity of mechanical stresses at the fracture line, but generally speaking the rate of proliferation does not reach the degree necessary for cartilage formation unless the bony fragments are pressed against each other and are free to move, so giving rise to shearing stresses across the fracture line.

The influence of cartilage implants on repair of the articular surface following experimental excision. By D. ALLBROOK and W. H. KIRKALDY-WILLIS. *Makerere College, The University College of East Africa*

Two series of experiments were performed. The objects were: (1) to elucidate the histological sequence of healing of the raw bony surface remaining after excision of one articular surface in a diarthrodial joint; and (2) to test the possible modifying influence on this of absorbable fixed cartilage implants capping the raw surface.

The radiohumeral joint of the vervet monkey was used, and after excision of the radial head histological changes were followed from 1 week to 2 years from operation. The main features of the reparative process were illustrated. Attention was called to the importance of the periosteogenic fibroblasts. These cells grew centripetally and covered the periphery of the severed bone end. Cells capping cut bone spicules lay down quantities of hyaline cartilage. This in turn became ossified and formed a plate of new cortical bone sealing the

underlying bone from the capping fibroblasts and joint space. The remaining hyaline cartilage, fibroblasts and collagen fibres now functioned as the new articular surface covering the new cortical bone plate.

Implants of whole cartilage, and fixed homo- and autotransplants were used to give a temporary articular surface. The same fundamental histological sequence was seen beneath the implant. This was gradually encroached upon by the capping fibroblasts but appeared to allow a more complete coverage of the raw bony surface and hence a better final articular surface.

Autografts of ileum to ear in the rabbit. By J. JOSEPH.
Guy's Hospital Medical School, London

Some skin from the inside of the ear was removed and a graft of ileum with the mucous membrane outwards was placed on the denuded area. Dressings were applied and after 10 days were removed. The ears were left undressed for subsequent periods (10–60 days).

In most animals the columnar epithelium was frequently in the form of cysts, where the lining epithelium was frequently flattened, or of a layer of surface epithelium leading into crypts beneath the growing skin. The ileal muscle invariably survived and showed an increase in some cases.

Frequently the cartilage of the ear showed a proliferation due to trauma, but in many specimens there was evidence of the formation of new cartilage and in three experiments bone developed.

In four specimens, after 10 and 30 days, the growing edge of the skin showed mitoses. This very unexpected finding prompted the search for mitoses in the growing edge of the skin of the inside of the ear following removal without grafting and these were readily found.

Some physical characters of the Nilotic tribes of the Sudan. By H. BUTLER.
University of Khartoum

The Nilotic tribes of the Sudan (Shilluk, Dinka and Nuer) are noted for their tall slender build and marked dolicocephaly. According to Seligman (1932) the average cephalic indices are: Shilluk 71.3, Dinka 72.7 and Nuer 73.5. Recent measurements of forty-two male Dinka from Bentiu give an average of 68.0 (range 62.4–76.6) and of 109 male Nuer at Fangak, an average of 69.7 (range 62.4–79.0). Four adult male Dinka skulls have cranial indices of 64.2, 64.3, 65.6 and 69.4; one adult male Nuer skull has an index of 66.6. The typical features of a Dinka skull are shown. It is suggested that the Nilotic tribes exhibit a greater degree of dolicocephaly than heretofore recognized. No other living African race shows such extreme dolicocephaly but it is equalled in the skeletal remains of the tall Mesolithic and Neolithic peoples of East Africa.

Measurements of the long bones of two male skeletons (one Dinka, one Nuer) show a relatively long lower limb (intermembranal indices are 67.2 and 68.7). The distal segment of both limbs, particularly the lower limb, is relatively very long. The combination of ultra-dolicocephaly and relatively great length of leg appears to be unique among living varieties of mankind.

Occipital curvature as an indication of physical affinities in Africa. By D. ALLBROOK.
Makerere College, The University College of East Africa

Tobias has recently contrasted the occipital curvature in Bushmen crania with that of African negroids. The former have a double occipital curve giving a bun-shaped outline, whereas the negroid has a single curve. The percentage ratio of the lambda-opisthion chord and arc is 81.2% in male Bushmen, and 84.9% in a group of S. African negroids.

Modern East African tribes at the northern limit of the Bantu linguistic area have resulted from the miscegenation of a number of different physical types. Analysis of the

distribution of this cranial feature might therefore present a clue to the influence of the Bushman stock in the modern population.

(1) In 100 male East African crania the usual pattern is a single curved occipital bone. The average occipital chord-arc index is 84·5 %. There is a distinct group of 10 % characterized by an index lying between 77 % and 80 %, having the classical bushmanoid bun-shaped occipital outline.

(2) In sixty male Abyssinian crania examined by permission of Prof. Sergi the average index is 83·2 %. The usual form is a single occipital curve. But 13 % show the bushmanoid occipital bun, and have an index between 77·5 % and 80 %.

(3) Seven fossilized crania extracted from tumulus burials scattered over central Tanganyika all have bushmanoid features. Where present the occipital bone has a well-marked bun and the type specimen has an occipital chord-arc index of 82 %.

This evidence indicates that the Bushman physical type has been at one time widespread throughout East and North-East Africa, and has played a significant part in the physical composition of the modern population.

Sexual characteristics of East African pelvic skeletons. By D. ALLEGROOK.
Makerere College, University College of East Africa

The frequency with which obstruction complicates labour has prompted the study of the pelvic configuration in the Lacurtine Bantu tribes of Uganda. X-ray pelvimetry of seventy patients combined with pelvimetry of thirty-one green and dried female pelvis demonstrates that the predominant type is the small, gynaecoid, shallow, generally contracted pelvis rather than the android type previously described.

Pelvimetry and inspection of articulated male specimens shows relatively slight differences from the females in many cases, and in these it is remarkably difficult to diagnose the sex with certainty. A clear sort can, however, be obtained by using indices based on the known greater length of the pubic bone in females proportional to the general size of the pelvis.

This local African pattern assumes new significance when skeletal form is viewed as the permanent memorial to the endocrine and nutritional state of its former owner.

The blood supply of the median nerve. By J. BLUNT.
St Bartholomew's Hospital Medical College, London

The blood supply of the median nerve in the forearm and hand has been investigated by injection methods and by the sodium nitroprusside-benzidine technique. The results indicate that, although subject to considerable minor variation, the main features of median nerve vascular anatomy are constant. In the upper and middle thirds of the forearm, branches of the median artery reach the nerve; immediately above the flexor retinaculum branches are supplied by the ulnar artery, and in the palm branches are received from the superficial palmar arch. In the cubital fossa the supply of arterial branches to the nerve is less constant, while at the wrist and in the hand, inconstant branches of supply are derived directly or indirectly from the radial artery. Veins draining the nerve accompany the corresponding nutrient arteries. The intrinsic vascular anatomy of the median nerve is similar to that of other peripheral nerve trunks, and is characterized by a well-marked interfascicular plexus. The results of the present study are discussed in relation to the findings of previous investigators and the clinical implications thereof are briefly considered.

The applied anatomy of venous collaterals in the lower limb. By E. MAVOR. *University of Aberdeen*

In deep vein thrombosis affecting the lower limbs the morbidity expected from the disease depends on the extent of the thrombosis and the nature of the collateral circulation. The correlation of clinical and venographic findings indicates that with thrombosis limited

to below the profunda femoris termination, the collateral circulation is adequate and thus morbidity minimal. In such circumstances important collaterals feed the profunda and internal saphenous systems. Even when the thrombosis is more extensive, the venae comitantes of the femoral artery may play a significant role in limiting morbidity. If these different venous collateral systems are unable to function because of high illo-femoral thrombosis, the venous insufficiency is severe. The collateral circulation has then failed as the main stem veins of the limb cannot be refilled proximally and the venous return in such cases may flow mainly to the contralateral internal iliac and long saphenous systems.

A series of venograms in the living subject is shown to illustrate the foregoing.

Arterial patterns in the rat spleen. By J. L. BRAITHWAITE, D. J. ADAMS and R. O. JONES. *University of Liverpool*

The arterial supply of the spleen has been studied in (i) the normal animal, (ii) in animals bearing experimental tumours, and (iii) in animals following ligation of either the central or the polar vascular pedicles.

The arteriograms following injection of a radiopaque medium show the typical 'dead tree' appearance in the normal animal. In those with slowly growing tumours there is a definite hyperaemia demonstrated by an increase in filling of the finest vessels in the spleen. When the blood supply to a rapidly growing tumour is cut off there is a marked perifollicular hyperaemia. Similar appearances to the latter are seen 4-7 days after ligation of the central vascular pedicle, although after 7 days with the ingrowth of newly formed vessels into the infarcted area this perifollicular hyperaemia disappears. The fact that injection medium can be introduced into the perifollicular space after initial injections of amyl nitrite and dibenamine suggests that there is some sphincteric control of the follicular vessels. The significance of this is discussed.

Morphological changes in the genital tract of the rat following interference with the gonadal blood supply. By W. R. M. MORTON. *The Queen's University, Belfast*

The macro- and microscopic appearances and the blood supply of the gonads and other parts of the genital tract have been investigated in adult male rats in which the internal spermatic vessels of both sides had been ligated and cut at ages varying from 49 to 84 days. Nine out of twenty such animals proved fertile; nine showed evidence of having mated but failed to procure young; and two failed to mate with normal adult females. Six of the fertile males had spermatozoa in both right and left genital tracts post mortem, two had them in the right and one in the left tract only; one of the non-fertile males had some sperms in both epididymides and another had them on the left side only; and in the remaining animals spermatozoa were absent from both sides. The vesiculae seminales and coagulating glands of the animals were not greatly different in appearance from those of normal animals. Disturbance of the gonad from its bed in the scrotum seemed to play some part in upsetting the development of the testes, as in almost all cases the testicular arteries filled with radio-opaque solution injected into the descending thoracic aorta, even when the testis was small and atrophic.

Types of prehensile hand. By R. W. HAINES. *Royal Medical College, Baghdad*

Correlation of the external and internal structure and what is known of the function of the hand in various mammals has allowed a more precise classification than has hitherto been possible. Convergent hands, in which the digits fall together as they are flexed, but have no special muscular arrangement to draw them together, appear to be the most primitive type found in placental mammals (all Paleocene forms known, *Herpestes*). Clasping hands have the contrahentes I and V arising from a common raphe and crossing the hand to the marginal digits (*Didelphys*, *Tatusia*). When the pollex is opposable to the

other digits it is usually provided with a nail rather than a claw, the thenar and first interdigital pads are usually approximated or fused and contrahens I is enlarged (*Dromicius*, *Cebus*). In schizodactylous hands digits I and II can oppose the others (*Phalanger*, *Lagothrix*). A few prehensile hands do not fall into this scheme, as *Tupaia*, where there is a common raphe for contrahentes II and V with a separate muscle for I, and *Coenolestes* where both digits I and V bear nails. What is known of the systematic and temporal distribution of these hand-types suggests a terrestrial rather than an arboreal ancestry for the placental mammals.

The lobe of the azygos vein. By D. BROWN. *Charing Cross Hospital Medical School, London*

The lobe of the azygos vein in the human lung is stated, variously, to be visible in between one in a hundred and one in a thousand human chest films. Several specimens possessing this abnormality have been obtained, from which casts have been prepared. By observing the order of bronchial branching occurring beneath the azygos fissure, it has been possible to assess the exact sequence of events leading to the occurrence of this abnormality. The significance of these findings is discussed with reference to the known pathology of the azygos lobe.

The canalicular system of bone. By J. H. MULLIGAN. *St Salvator's College, University of St Andrews*

The results obtained by a silver technique for the demonstration of the cement lamellae and the canalicular system of bone have been compared with those given (a) by staining, (b) by the air inclusion method of Flemming, and (c) by Schmorl's thionin and pierie acid method.

Teleology and the intervertebral disc. By M. G. C. HENDRY. *University of Aberdeen*

The ultrastructure of the nucleus pulposus has become known only in recent years. The information now available throws light on the function of the normal disc, in particular on the mechanism of water retention in the nucleus. The supposition that the nucleus maintains its hydration by osmosis is incorrect: good grounds can be shown for the belief that the imbibition pressure of the protein/polysaccharide gel of the nucleus is instead responsible. On the basis of this concept, both annulus and nucleus can be envisaged as playing an active role in the transmission of forces, and an adequate physico-chemical explanation is provided of the ability of the normal disc to withstand very large stresses.

A disturbance of the physics of this process has been demonstrated in discs which have been the site of a mechanical derangement. Work is now proceeding on the chemical basis underlying these changes, and on the coincident alterations in the ultrastructure of the nucleus. These studies throw some light on the probable aetiology of disc derangement.

The pelvis of the rat: sexual dimorphism and the effects of castration.
By T. J. HARRISON. *The Queen's University, Belfast*

Twenty male Wistar rats were castrated between the 40th and 50th days of life and their pelvic dimensions during growth compared with those of nineteen normal male litter-mates. Twelve females were spayed at a similar age and their pelvic dimensions compared with those of twelve normal female litter-mates. The pelvis of each rat was radiographed at the 10th day of life and at uniform intervals up to 1 year. The pelvic dimensions during growth were measured on the radiographs. The rats were killed at 1 year and the pelves cleaned of soft parts, stained with alizarin, and cleared in glycerine. Further measurements (e.g. symphyseal length) were made on these specimens. Growth curves of innominate bone length, sacral length and sacral breadth were constructed for individual rats in each litter.

All the measurements at 1 year on the control male pelvis were statistically highly significantly greater than those of the control females ($P < 0.001$). The upper margin of the symphysis lay at a lower vertebral level in the female. Castration had no significant effect on pelvic growth in the male rat. In the female the lengths of innominate bone, sacrum, and symphysis were significantly greater in the spayed animal ($P < 0.05$).

The evolution of some mammalian and avian traction epiphyses from sesamoid bones. By C. H. BARNETT and O. J. LEWIS. *St Thomas's Hospital Medical School, London*

Parsons (*J. Anat., Lond.*, **38**, 1904) has used the term 'traction epiphysis' to denote a bony projection to which a tendon is attached and which has an independent centre of ossification. He has produced evidence that such an epiphysis may represent a former sesamoid, now fused to the bone against which it rubbed, but his theory has received little support.

A number of sesamoids commonly present at the knee, elbow and heel region have been examined. The occurrence in a few animals of traction epiphyses corresponding in form and position to these sesamoids indicates that one type of structure has probably given rise to the other.

Developmental stages of the following traction epiphyses have been studied: the tibial crest of birds and the greater tuberosity of the humerus, the greater trochanter of the femur and the fibular process of certain mammals. The embryology of all these epiphyses suggests that they were formerly independent of the bone to which they now belong.

The present findings accord with the theory advanced by Parsons rather than with the contrary view, argued by Pearson and Davin (*Biometrika*, **13**, 1921), that traction epiphyses are the precursors of sesamoid bones.

A case of trifid ureter with one branch ending blindly.

By W. K. J. WALLS. *University of Leeds*

This anomaly was a chance finding in a male cadaver aged 82 years. Although multiple or divided ureters are not uncommon, blind endings of such ureters are comparatively rarely reported in the literature.

The left kidney showed a double pelvis connected to two branches of the trifid ureter. During its descent the branch connected to the upper part of the kidney crossed in front of that connected to the lower part to gain its lateral side and the two then descended together to join one inch before reaching the bladder. The third branch lay medial to the other two and had a blind upper end at the level of the pelvic brim: it joined the branch draining the lower part of the kidney two inches from the bladder. The trifid ureter opened into the bladder by a single orifice. The right kidney and ureter appeared normal.

The effects of X-rays and radiomimetic substances on the solid concentration of lymphocytes. By R. BARER and S. JOSEPH. *University of Oxford*

Normal mice were treated with X-ray doses of 300 r. and tail blood samples were examined over periods of 28 days. The cytoplasmic concentration of the lymphocytes was estimated by refractometry (Barer, *Physical Techniques in Biological Research*, **3**, New York, 1956). There was a rapid initial fall in concentration, evident within ten minutes. Recovery began after about 2 days. There was usually a rise to values slightly above normal in 5–8 days, followed by a slight secondary fall (9–13 days) and a further recovery peak at 15–18 days with a gradual return to normal values after 24 days. Injection of the Nitrogen Mustard derivative CB 1348 gave similar results. These changes showed a remarkable parallelism with the variations in lymphocyte count (Elson, *Symposium on Radiobiology (Liège)*, London, 1954). It was suggested that the first recovery peak represented the entry of a new cell population into the blood stream. The secondary fall may

be caused by latent toxins or by the failure of the damaged lymphatic organs to maintain the output of new cells. The delayed rise at 15–18 days suggested the production of more new cells and may be related to the claim (Fichtelius, *Acta Anat.* 31, 1957) that lymphocytes labelled with radioactive isotopes appeared in the circulation in two separate phases.

This work was assisted by the British Empire Cancer Campaign.

Postnatal changes in the histology of the coagulating gland in the rat.

By E. J. CLEGG. *University of Liverpool*

The histology of the accessory reproductive organs has been studied in nineteen animals aged between 1 and 44 days.

By the age of 5 days canalization of the coagulating gland and its duct has begun and by 21 days about two-thirds of the acini have lumina. The process is completed by the 30th day in all cases.

At 21 days about a quarter of the acini having lumina contain cellular debris; this proportion increases up to the 30th day and thereafter diminishes. Occasional debris-containing acini can be seen at 44 days. Such acini frequently exhibit evidence of necrosis of the epithelial lining. Mild cases merely show irregularity and density of the nucleus and increased eosinophilia of the cytoplasm, but more severe changes are evidenced by necrosis of the tips of villi and partial or complete shedding of the epithelium into the lumina.

It is considered that a relative vascular inefficiency may be responsible for these appearances.

The nail bed in normal human subjects and in those showing 'splinter haemorrhages of the nail'. By B. F. MARTIN and M. M. PLATTS. *University of Sheffield*

The terminal segment of a normal finger was obtained from each of ten cadavers at post-mortem, and one from each of nine cadavers which showed 'splinter haemorrhages of the nail'. After fixation in Heidenhain's 'Susa' and removal of the phalanx, the finger ends were embedded in paraffin, sectioned longitudinally, and stained with routine and special stains. The histological features of the normal nail bed were studied in detail and comparison made with the features shown in cases of splinter haemorrhage, particular attention being directed to the blood vessels.

It was found that the so-called splinter haemorrhages gave positive staining reactions for haemoglobin, indicating that they were in fact actual haemorrhages, and they occurred in a specific part of the hyponychium, namely, in the angle between the nail plate and the terminal part of the stratum granulosum. In most specimens examined, there was considerable blood stasis in the vessels of the nail bed and finger tip, and the vessels supplying the dermal papillae in the region of the haemorrhage were grossly dilated and tortuous, whereas the corresponding vessels in other parts of the nail bed and finger tip were not, indicating the existence of critical vascular points at which the haemorrhages occurred.

The ultrastructure of the parietal cells of the stomach in the mouse.

By A. D. HALLY. *University of Glasgow*

The parietal cells in the stomach of the mouse have been examined with the electron microscope. The most prominent feature in the parietal cell is the intracellular canals, which are lined with numerous microvilli. The intercellular canals are clefts between adjacent mucous neck cells or chief cells lined with sparse microvilli: they are thus similar to the biliary canaliculi. The mitochondria, which are large and ovoid, occupy far more of the cytoplasm than in other cells. Their internal structure shows closely packed cristae.

Numerous discrete small vesicles lie in the cytoplasm and extend to the surface of the

intracellular canals. Although these vesicles are often contiguous they do not anastomose and thus do not form a reticulum. There are a few scattered short tubules of granular endoplasmic reticulum, and Palade granules occur in small clusters. There is no typical Golgi complex as is seen in most other secretory cells. The findings are discussed in relation to the function of the cell.

A histochemical and electron microscopic study of the thyroid gland in the normal and hypophysectomized rat. By J. D. LEVER. *University of Cambridge*

Biopsy specimens of rat thyroid gland were examined as a normal control series. The animals were then hypophysectomized and subsequently sacrificed after either 10 or 28 days: the remaining thyroid tissue and one adrenal gland were then removed. Body weight loss and certain histological changes in the adrenal cortex were taken as evidence of successful hypophysectomy. Thyroid tissues were investigated by the following means: (i) galloxyanin stain for nucleic acids, some material being pretreated with ribonuclease; (ii) PAS reaction for colloid demonstration; (iii) α -naphthol staining reaction for peroxidase; (iv) electron microscopy.

Staining for peroxidase was most intense within cytoplasmic bodies ranging in size from 0.5–1.25 μ . In electron micrographs, intracellular bodies of comparable size and distribution contained dense 80 Å particles, in some of which smaller 10–15 Å components were detected. The possible significance of these findings was discussed. Following hypophysectomy there was a decline in cytoplasmic nucleic acid and in the number of intracellular colloid bodies; also an obvious reduction in surface area of the endoplasmic reticulum and in the number of cytoplasmic Palade granules.

A histochemical study of the nasal gland of the duck.
By R. J. SCOTHORNE. *University of Glasgow*

The nasal ('supraorbital') gland of the duck has been studied by a variety of histological and histochemical techniques. These demonstrate the complete absence of mucus-secreting elements (PAS, toluidine blue, alcian blue); paucity of cytoplasmic R.N.S. (methyl-green/pyronin, toluidine blue) and absence of 'zymogen' granules; abundant phospholipid material throughout the secretory elements (Sudan black, Baker's acid haematein); remarkably abundant mitochondria; the presence of alkaline phosphatase, confined to the extreme periphery of each secretory lobule. These findings are discussed in relation to the various functions which have been ascribed to the nasal glands of birds: that they produce a 'slimy' secretion (? mucus) by the holocrine method (Marples, 1932); that they produce a 'serous' secretion (various authors); and that, in marine birds, they secrete sodium chloride in quantities much greater than those produced by the kidney (Schmidt-Nielsen & Sladen, 1958).

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